

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
11 April 2002 (11.04.2002)

PCT

(10) International Publication Number
WO 02/28831 A1

(51) International Patent Classification⁷: **C07D 209/12,**
A61K 31/4045

Rahway, NJ 07065-0907 (US). **SISKO, John, T.** [US/US];
126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(21) International Application Number: **PCT/US01/42389**

(74) Common Representative: **MERCK & CO., INC.**; 126
East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(22) International Filing Date:
28 September 2001 (28.09.2001)

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK,
LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX,
MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK,
SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA,
ZW.

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/237,241 2 October 2000 (02.10.2000) US

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,
CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD,
TG).

(71) Applicant (*for all designated States except US*): **MERCK
& CO., INC.** [US/US]; 126 East Lincoln Avenue, Rahway,
NJ 07065-0907 (US).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **TROTTER, B.,
Wesley** [US/US]; 126 East Lincoln Avenue, Rahway, NJ
07065-0907 (US). **QUIGLEY, Amy, G.** [US/US]; 126
East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
DINSMORE, Christopher, J. [US/US]; 126 East Lin-
coln Avenue, Rahway, NJ 07065-0907 (US). **LUMMA,
William, C., Jr.** [US/US]; 126 East Lincoln Avenue,

Published:

— with international search report

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*



WO 02/28831 A1

(54) Title: **INHIBITORS OF PRENYL-PROTEIN TRANSFERASE**

(57) Abstract: The present invention is directed to fused bicyclic compounds which inhibit prenyl-protein transferase and the prenylation of the oncogene protein Ras. The invention is further directed to chemotherapeutic compositions containing the compounds of this invention and methods for inhibiting prenyl-protein transferase and the prenylation of the oncogene protein Ras.

BEST AVAILABLE COPY

TITLE OF THE INVENTION INHIBITORS OF PRENYL-PROTEIN TRANSFERASE

BACKGROUND OF THE INVENTION

5 The Ras proteins (Ha-Ras, Ki4a-Ras, Ki4b-Ras and N-Ras) are part of a signalling pathway that links cell surface growth factor receptors to nuclear signals initiating cellular proliferation. Biological and biochemical studies of Ras action indicate that Ras functions like a G-regulatory protein. In the inactive state, Ras is bound to GDP. Upon growth factor receptor activation Ras is induced to exchange
10 GDP for GTP and undergoes a conformational change. The GTP-bound form of Ras propagates the growth stimulatory signal until the signal is terminated by the intrinsic GTPase activity of Ras, which returns the protein to its inactive GDP bound form (D.R. Lowy and D.M. Willumsen, *Ann. Rev. Biochem.* 62:851-891 (1993)). Mutated *ras* genes (Ha-*ras*, Ki4a-*ras*, Ki4b-*ras* and N-*ras*) are found in many human
15 cancers including colorectal carcinoma, exocrine pancreatic carcinoma, and myeloid leukemias. The protein products of these genes are defective in their GTPase activity and constitutively transmit a growth stimulatory signal.

 Ras must be localized to the plasma membrane for both normal and oncogenic functions. At least 3 post-translational modifications are involved
20 with Ras membrane localization, and all 3 modifications occur at the C-terminus of Ras. The Ras C-terminus contains a sequence motif termed a "CAAX" or "Cys-Aaa¹-Aaa²-Xaa" box (Cys is cysteine, Aaa is an aliphatic amino acid, the Xaa is any amino acid) (Willumsen *et al.*, *Nature* 310:583-586 (1984)). Depending on the specific sequence, this motif serves as a signal sequence for the enzymes farnesyl-
25 protein transferase or geranylgeranyl-protein transferase type I, which catalyze the alkylation of the cysteine residue of the CAAX motif with a C15 or C20 isoprenoid, respectively. (S. Clarke., *Ann. Rev. Biochem.* 61:355-386 (1992); W.R. Schafer and J. Rine, *Ann. Rev. Genetics* 30:209-237 (1992)). The term prenyl-protein transferase
30 may be used to refer generally to farnesyl-protein transferase and geranylgeranyl-protein transferase type I. The Ras protein is one of several proteins that are known to undergo post-translational farnesylation. Other farnesylated proteins include the Ras-related GTP-binding proteins such as Rho, fungal mating factors, the nuclear lamins, and the gamma subunit of transducin. James, et al., *J. Biol. Chem.* 269, 14182 (1994) have identified a peroxisome associated protein Pxf which is also farnesylated.

James, et al., have also suggested that there are farnesylated proteins of unknown structure and function in addition to those listed above.

Inhibition of farnesyl-protein transferase has been shown to block the growth of Ras-transformed cells in soft agar and to modify other aspects of their transformed phenotype. It has also been demonstrated that certain inhibitors of farnesyl-protein transferase selectively block the processing of the Ras oncoprotein intracellularly (N.E. Kohl et al., *Science*, 260:1934-1937 (1993) and G.L. James et al., *Science*, 260:1937-1942 (1993). Recently, it has been shown that an inhibitor of farnesyl-protein transferase blocks the growth of *ras*-dependent tumors in nude mice (N.E. Kohl et al., *Proc. Natl. Acad. Sci U.S.A.*, 91:9141-9145 (1994) and induces regression of mammary and salivary carcinomas in *ras* transgenic mice (N.E. Kohl et al., *Nature Medicine*, 1:792-797 (1995).

Indirect inhibition of farnesyl-protein transferase *in vivo* has been demonstrated with lovastatin (Merck & Co., Rahway, NJ) and compactin (Hancock et al., *ibid*; Casey et al., *ibid*; Schafer et al., *Science* 245:379 (1989)). These drugs inhibit HMG-CoA reductase, the rate limiting enzyme for the production of polyisoprenoids including farnesyl pyrophosphate. Farnesyl-protein transferase utilizes farnesyl pyrophosphate to covalently modify the Cys thiol group of the Ras CAAX box with a farnesyl group (Reiss et al., *Cell*, 62:81-88 (1990); Schaber et al., *J. Biol. Chem.*, 265:14701-14704 (1990); Schafer et al., *Science*, 249:1133-1139 (1990); Manne et al., *Proc. Natl. Acad. Sci USA*, 87:7541-7545 (1990)). Inhibition of farnesyl pyrophosphate biosynthesis by inhibiting HMG-CoA reductase blocks Ras membrane localization in cultured cells. However, direct inhibition of farnesyl-protein transferase would be more specific and attended by fewer side effects than would occur with the required dose of a general inhibitor of isoprene biosynthesis.

Inhibitors of farnesyl-protein transferase (FPTase) have been described in two general classes. The first are analogs of farnesyl diphosphate (FPP), while the second class of inhibitors is related to the protein substrates (e.g., Ras) for the enzyme. The peptide derived inhibitors that have been described are generally cysteine containing molecules that are related to the CAAX motif that is the signal for protein prenylation. (Schaber et al., *ibid*; Reiss et al., *ibid*; Reiss et al., *PNAS*, 88:732-736 (1991)). Such inhibitors may inhibit protein prenylation while serving as alternate substrates for the farnesyl-protein transferase enzyme, or may be purely competitive inhibitors (U.S. Patent 5,141,851, University of Texas; N.E. Kohl et al., *Science*, 260:1934-1937 (1993); Graham, et al., *J. Med. Chem.*, 37, 725 (1994)). In

general, deletion of the thiol from a CAAX derivative has been shown to dramatically reduce the inhibitory potency of the compound. However, the thiol group potentially places limitations on the therapeutic application of FPTase inhibitors with respect to pharmacokinetics, pharmacodynamics and toxicity. Therefore, a functional replacement for the thiol is desirable.

It has recently been reported that farnesyl-protein transferase inhibitors are inhibitors of proliferation of vascular smooth muscle cells and are therefore useful in the prevention and therapy of arteriosclerosis and diabetic disturbance of blood vessels (JP H7-112930).

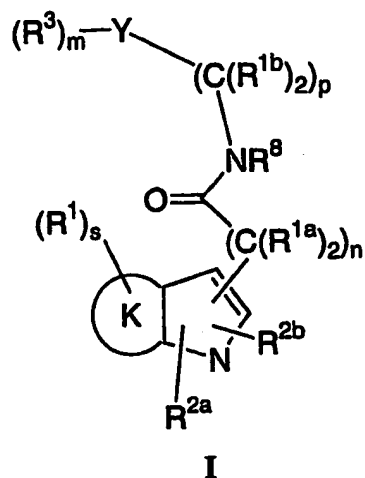
It has recently been disclosed that certain tricyclic compounds which optionally incorporate a piperidine moiety are inhibitors of FPTase (WO 95/10514, WO 95/10515 and WO 95/10516). Imidazole-containing inhibitors of farnesyl protein transferase have also been disclosed (WO 95/09001 and EP 0 675 112 A1).

It is, therefore, an object of this invention to develop compounds that do not have a thiol moiety, and that will inhibit prenyl-protein transferase and thus, the post-translational prenylation of proteins. It is a further object of this invention to develop chemotherapeutic compositions containing the compounds of this invention and methods for producing the compounds of this invention.

SUMMARY OF THE INVENTION

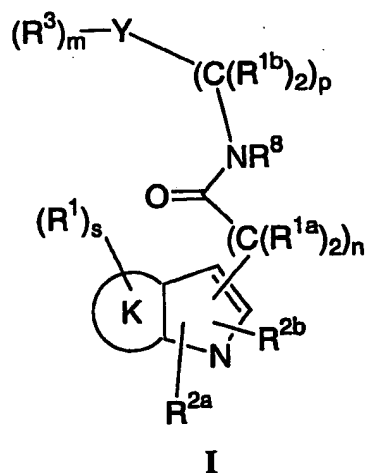
The present invention comprises fused bicyclic compounds which inhibit prenyl-protein transferase. Further contained in this invention are chemotherapeutic compositions containing these prenyl-protein transferase inhibitors and methods for their production.

The compounds of this invention are illustrated by the formula I:

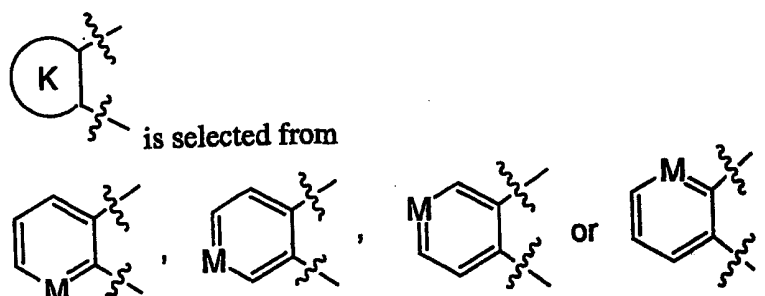


DETAILED DESCRIPTION OF THE INVENTION

- The compounds of this invention are useful in the inhibition of prenyl-
 5 protein transferase and the prenylation of the oncogene protein Ras. In a first
 embodiment, the compounds of the instant invention are illustrated by the formula I:



wherein



M is C or N;

5

R¹ is independently selected from

- a) H,
 b) unsubstituted or substituted C₁-C₆ alkyl,
 c) unsubstituted or substituted C₁-C₆ alkoxy,
 10 d) unsubstituted or substituted aryl,
 e) unsubstituted or substituted C₂-C₈ alkenyl,
 f) unsubstituted or substituted C₂-C₈ alkynyl,
 g) unsubstituted or substituted perfluoroalkyl,
 h) halo,
 15 i) OR¹⁰,
 j) R¹¹S(O)_m,
 k) R¹⁰C(O)NR¹⁰-,
 l) -C(O)N(R¹⁰)₂,
 m) CN,
 20 n) NO₂,
 o) R¹⁰C(O)-,
 p) R¹⁰OC(O)-,
 q) N₃,
 r) -N(R¹⁰)₂, or
 25 s) R¹¹OC(O)NR¹⁰-;

R^{1a} and R^{1b} are independently selected from:

- a) H,
 b) unsubstituted or substituted C₁-C₆ alkyl,

- 5
- c) unsubstituted or substituted C₁-C₆ alkoxy,
 - d) unsubstituted or substituted aryl,
 - e) unsubstituted or substituted heterocycle,
 - f) unsubstituted or substituted aralkyl, or
 - g) -(CH₂)_n heterocycle;

R_{2a} and R_{2b} are independently selected from:

- 10
- a) H,
 - b) unsubstituted or substituted C₁-C₆ alkyl,
 - c) unsubstituted or substituted aryl,
 - d) oxo,
 - e) unsubstituted or substituted heterocycle, or
 - f) unsubstituted or substituted C₁-C₆ alkoxy;

wherein the substituted group is substituted with from one to three substituents
selected from:

- 15
- 1) unsubstituted or substituted C₁-C₆ alkyl,
 - 2) unsubstituted or substituted C₁-C₆ alkoxy,
 - 3) halo,
 - 4) unsubstituted or substituted aryl,
 - 20
 - 5) unsubstituted or substituted heterocycle,
 - 6) unsubstituted or substituted C₂-C₈ alkenyl,
 - 7) unsubstituted or substituted C₂-C₈ alkynyl,
 - 8) unsubstituted or substituted perfluoroalkyl,
 - 9) -OR¹⁰,
 - 25
 - 10) R¹¹S(O)_m-,
 - 11) R¹⁰C(O)NR¹⁰-,
 - 12) -C(O)N(R¹⁰)₂,
 - 13) CN,
 - 14) NO₂,
 - 30
 - 15) -N(R¹⁰)₂,
 - 16) R¹⁰C(O)-,
 - 17) R¹⁰OC(O)-,
 - 18) N₃, or
 - 19) R¹¹OC(O)NR¹⁰-;

R2a and R2b may optionally be joined in a ring;

R3 is independently selected from:

- 5 a) H,
- b) unsubstituted or substituted C₁-C₆ alkyl,
- c) unsubstituted or substituted C₂-C₈ alkenyl,
- d) unsubstituted or substituted C₂-C₈ alkynyl,
- e) unsubstituted or substituted perfluoroalkyl,
- 10 f) halo,
- g) -OR¹⁰,
- h) R¹¹S(O)_m-,
- i) R¹⁰C(O)NR¹⁰-,
- j) -C(O)NR¹⁰,
- 15 k) CN,
- l) NO₂,
- m) R¹⁰C(O)-,
- n) R¹⁰OC(O)-,
- o) N₃,
- 20 p) -N(R¹⁰)₂, or
- q) R¹¹OC(O)NR¹⁰-;

R⁸ is selected from:

- 25 a) unsubstituted or substituted C₁-C₆ alkyl,
- b) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
- c) unsubstituted or substituted aryl,
- d) unsubstituted or substituted C₂-C₈ alkenyl, or
- e) unsubstituted or substituted C₂-C₈ alkynyl;

wherein the substituted group is selected from:

- 30 1) unsubstituted or substituted C₁-C₆ alkyl,
- 2) unsubstituted or substituted C₂-C₈ alkenyl,
- 3) unsubstituted or substituted C₂-C₈ alkynyl,
- 4) unsubstituted or substituted C₁-C₆ alkoxy,
- 5) CN,
- 35 6) unsubstituted or substituted aryl, or

7) unsubstituted or substituted heterocycle;

R¹⁰ is selected from:

- a) H,
- 5 b) unsubstituted or substituted C₁-C₆ alkyl,
- c) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
- d) unsubstituted or substituted aryl,
- e) unsubstituted or substituted heterocycle,
- f) unsubstituted or substituted aralkyl, or
- 10 g) unsubstituted or substituted heterocyclalkyl;

R¹¹ is selected from

- a) unsubstituted or substituted C₁-C₆ alkyl,
- b) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
- 15 c) unsubstituted or substituted aryl,
- d) unsubstituted or substituted heterocycle,
- e) unsubstituted or substituted aralkyl, or
- f) unsubstituted or substituted heterocyclalkyl;

20 Y is selected from:

- a) heterocycle,
- b) C₃-C₁₀ cycloalkyl,
- c) C₂-C₈ alkenyl,
- d) C₂-C₈ alkynyl,
- 25 e) C₁-C₆ alkoxy,
- f) CN,
- g) C(O),
- h) C(O)OR¹⁰,
- i) -OR¹⁰,
- 30 j) -N(R¹⁰)₂, or
- k) C(=NH)NHR¹⁰;

m is 0 to 4;

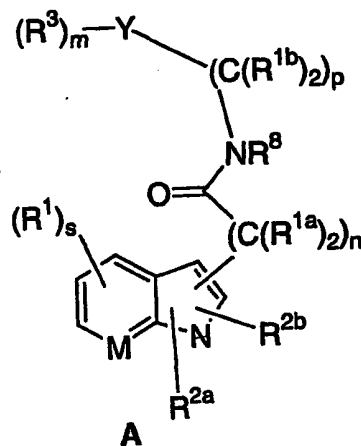
n is 0 to 4;

35 p is 1 to 4;

s is 0 to 4;

or a pharmaceutically acceptable salt or stereoisomer thereof.

5 In a further embodiment of this invention, the inhibitors of prenyl-protein transferase are illustrated by the formula A:



10 wherein

M is C or N;

R¹ is independently selected from

- 15 a) H,
- b) unsubstituted or substituted C₁-C₆ alkyl,
- c) unsubstituted or substituted C₁-C₆ alkoxy,
- d) unsubstituted or substituted aryl,
- e) unsubstituted or substituted C₂-C₈ alkenyl,
- 20 f) unsubstituted or substituted C₂-C₈ alkynyl,
- g) unsubstituted or substituted perfluoroalkyl,
- h) halo,
- i) OR¹⁰,
- j) R¹¹S(O)_m,
- 25 k) R¹⁰C(O)NR¹⁰-,
- l) -C(O)N(R¹⁰)₂,

- 5
- m) CN,
 - n) NO₂,
 - o) R¹⁰C(O)-,
 - p) R¹⁰OC(O)-,
 - q) N₃,
 - r) -N(R¹⁰)₂, or
 - s) R¹¹OC(O)NR¹⁰-;

R^{1a} is independently selected from:

- 10
- a) H,
 - b) unsubstituted or substituted C₁-C₆ alkyl,
 - c) unsubstituted or substituted C₁-C₆ alkoxy,
 - d) unsubstituted or substituted aryl, or
 - e) unsubstituted or substituted heterocycle;

15

R^{1b} is independently selected from:

- 20
- a) H,
 - b) unsubstituted or substituted C₁-C₆ alkyl,
 - c) unsubstituted or substituted C₁-C₆ alkoxy,
 - d) unsubstituted or substituted aryl,
 - e) unsubstituted or substituted heterocycle,
 - f) unsubstituted or substituted aralkyl, or
 - g) -(CH₂)_n heterocycle;

25 R^{2a} and R^{2b} are independently selected from:

- 30
- a) H,
 - b) unsubstituted or substituted C₁-C₆ alkyl,
 - c) unsubstituted or substituted aryl,
 - d) oxo,
 - e) unsubstituted or substituted heterocycle, or
 - f) unsubstituted or substituted C₁-C₆ alkoxy;

wherein the substituted group is substituted with from one to three substituents selected from:

- 35
- 1) unsubstituted or substituted C₁-C₆ alkyl,
 - 2) unsubstituted or substituted C₁-C₆ alkoxy,

- 3) halo,
- 4) unsubstituted or substituted aryl,
- 5) unsubstituted or substituted heterocycle,
- 6) unsubstituted or substituted C₂-C₈ alkenyl,
- 7) unsubstituted or substituted C₂-C₈ alkynyl,
- 8) unsubstituted or substituted perfluoroalkyl,
- 9) -OR¹⁰,
- 10) R¹¹S(O)_m-,
- 11) R¹⁰C(O)NR¹⁰-,
- 12) -C(O)N(R¹⁰)₂,
- 13) CN,
- 14) NO₂,
- 15) -N(R¹⁰)₂,
- 16) R¹⁰C(O)-,
- 17) R¹⁰OC(O)-,
- 18) N₃, or
- 19) R¹¹OC(O)NR¹⁰-;

R^{2a} and R^{2b} may optionally be joined in a ring;

20

R³ is independently selected from:

- a) H,
- b) unsubstituted or substituted C₁-C₆ alkyl,
- c) unsubstituted or substituted C₂-C₈ alkenyl,
- d) unsubstituted or substituted C₂-C₈ alkynyl,
- e) unsubstituted or substituted perfluoroalkyl,
- f) halo,
- g) -OR¹⁰,
- h) R¹¹S(O)_m-,
- i) R¹⁰C(O)NR¹⁰-,
- j) -C(O)NR¹⁰,
- k) CN,
- l) NO₂,
- m) R¹⁰C(O)-,
- n) R¹⁰OC(O)-,

- o) N_3 ,
- p) $-N(R^{10})_2$, or
- q) $R^{11}OC(O)NR^{10}$;

5 R^8 is selected from:

- a) unsubstituted or substituted C_1 - C_6 alkyl,
- b) unsubstituted or substituted C_3 - C_{10} cycloalkyl,
- c) unsubstituted or substituted aryl,
- d) unsubstituted or substituted C_2 - C_8 alkenyl, or
- 10 e) unsubstituted or substituted C_2 - C_8 alkynyl;

wherein the substituted group is selected from:

- 1) unsubstituted or substituted C_1 - C_6 alkyl,
- 2) unsubstituted or substituted C_2 - C_8 alkenyl,
- 3) unsubstituted or substituted C_2 - C_8 alkynyl,
- 15 4) unsubstituted or substituted C_1 - C_6 alkoxy,
- 5) CN ,
- 6) unsubstituted or substituted aryl, or
- 7) unsubstituted or substituted heterocycle;

20 R^{10} is selected from:

- a) H ,
- b) unsubstituted or substituted C_1 - C_6 alkyl,
- c) unsubstituted or substituted C_3 - C_{10} cycloalkyl,
- d) unsubstituted or substituted aryl,
- 25 e) unsubstituted or substituted heterocycle,
- f) unsubstituted or substituted aralkyl, or
- g) unsubstituted or substituted heterocyclalkyl;

R^{11} is selected from

- 30 a) unsubstituted or substituted C_1 - C_6 alkyl,
- b) unsubstituted or substituted C_3 - C_{10} cycloalkyl,
- c) unsubstituted or substituted aryl,
- d) unsubstituted or substituted heterocycle,
- e) unsubstituted or substituted aralkyl, or

f) unsubstituted or substituted heterocyclalkyl;

Y is selected from:

- a) heterocycle,
- 5 b) C₃-C₁₀ cycloalkyl,
- c) C₂-C₈ alkenyl,
- d) C₂-C₈ alkynyl,
- e) C₁-C₆ alkoxy,
- f) CN,
- 10 g) C(O),
- h) C(O)OR¹⁰,
- i) -OR¹⁰,
- j) -N(R¹⁰)₂, or
- 15 k) C(=NH)NHR¹⁰;

m is 0 to 4;

n is 0 or 1;

p is 1 to 4;

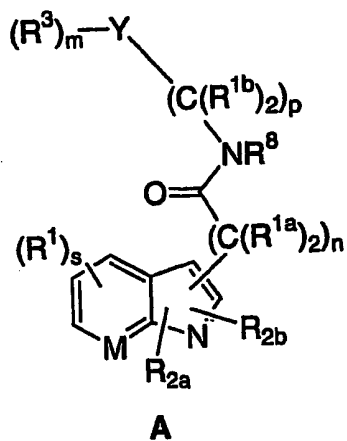
s is 0 to 4;

20

or a pharmaceutically acceptable salt or stereoisomer thereof.

In another embodiment of the instant invention, the inhibitors of prenyl-protein transferase are illustrated by the formula A:

25



wherein

M is C or N;

5 R¹ is independently selected from

- a) H,
- b) unsubstituted or substituted C₁-C₆ alkyl,
- c) unsubstituted or substituted C₁-C₆ alkoxy,
- d) unsubstituted or substituted aryl,
- 10 e) unsubstituted or substituted perfluoroalkyl,
- f) halo,
- g) OR¹⁰,
- h) R¹⁰C(O)NR¹⁰-,
- i) -C(O)N(R¹⁰)₂,
- 15 j) CN,
- k) R¹⁰C(O)-,
- l) R¹⁰OC(O)-,
- m) -N(R¹⁰)₂, or
- n) R¹¹OC(O)NR¹⁰-;

20

R^{1a} is independently selected from:

- a) H,
- b) unsubstituted or substituted C₁-C₆ alkyl, or
- c) unsubstituted or substituted C₁-C₆ alkoxy;

25

R^{1b} is independently selected from:

- a) H,
- b) unsubstituted or substituted C₁-C₆ alkyl,
- c) unsubstituted or substituted C₁-C₆ alkoxy,
- 30 d) unsubstituted or substituted aryl,
- e) unsubstituted or substituted heterocycle,
- f) unsubstituted or substituted aralkyl, or
- g) -(CH₂)_n heterocycle;

35 R^{2a} and R^{2b} are independently selected from:

- 5
- a) H,
 - b) unsubstituted or substituted C₁-C₆ alkyl,
 - c) unsubstituted or substituted aryl,
 - d) oxo,
 - e) unsubstituted or substituted heterocycle, or
 - f) unsubstituted or substituted C₁-C₆ alkoxy;

wherein the substituted group is substituted with from one to three substituents selected from:

- 10
- 1) unsubstituted or substituted C₁-C₆ alkyl,
 - 2) unsubstituted or substituted C₁-C₆ alkoxy,
 - 3) halo,
 - 4) unsubstituted or substituted aryl,
 - 5) unsubstituted or substituted heterocycle,
 - 6) unsubstituted or substituted perfluoroalkyl,
 - 15 7) -OR¹⁰,
 - 8) R¹⁰C(O)NR¹⁰-,
 - 9) -C(O)N(R¹⁰)₂,
 - 10) CN,
 - 11) NO₂, or
 - 20 12) -N(R¹⁰)₂;

R^{2a} and R^{2b} may optionally be joined in a ring;

R³ is independently selected from:

- 25
- a) H,
 - b) unsubstituted or substituted C₁-C₆ alkyl,
 - c) halo,
 - d) -OR¹⁰,
 - e) R¹¹S(O)_m-,
 - 30 f) R¹⁰C(O)NR¹⁰-,
 - g) -C(O)NR¹⁰,
 - h) R¹⁰C(O)-,
 - i) -N(R¹⁰)₂, or
 - j) R¹¹OC(O)NR¹⁰-;
- 35

R⁸ is selected from:

- 5
- a) unsubstituted or substituted C₁-C₆ alkyl,
 - b) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
 - c) unsubstituted or substituted aryl,
 - d) unsubstituted or substituted C₂-C₈ alkenyl, or
 - e) unsubstituted or substituted C₂-C₈ alkynyl,

wherein the substituted group is selected from:

- 10
- 1) unsubstituted or substituted C₁-C₆ alkyl,
 - 2) unsubstituted or substituted C₂-C₈ alkenyl,
 - 3) unsubstituted or substituted C₂-C₈ alkynyl,
 - 4) unsubstituted or substituted C₁-C₆ alkoxy,
 - 5) CN,
 - 6) unsubstituted or substituted aryl, or
 - 7) unsubstituted or substituted heterocycle;

15

R¹⁰ is selected from:

- 20
- a) H,
 - b) unsubstituted or substituted C₁-C₆ alkyl,
 - c) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
 - d) unsubstituted or substituted aryl,
 - e) unsubstituted or substituted heterocycle,
 - f) unsubstituted or substituted aralkyl, or
 - g) unsubstituted or substituted heterocyclalkyl;

25 R¹¹ is selected from

- 30
- a) unsubstituted or substituted C₁-C₆ alkyl,
 - b) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
 - c) unsubstituted or substituted aryl,
 - d) unsubstituted or substituted heterocycle,
 - e) unsubstituted or substituted aralkyl, or
 - f) unsubstituted or substituted heterocyclalkyl;

Y is selected from:

- a) heterocycle,

- b) C₃-C₁₀ cycloalkyl,
 c) C₂-C₈ alkenyl,
 d) C₂-C₈ alkynyl,
 e) C₁-C₆ alkoxy,
 5 f) CN,
 g) C(O),
 h) C(O)OR¹⁰,
 i) -OR¹⁰,
 j) -N(R¹⁰)₂, or
 10 k) C(=NH)NHR¹⁰;

m is 0 to 4;

n is 0 or 1;

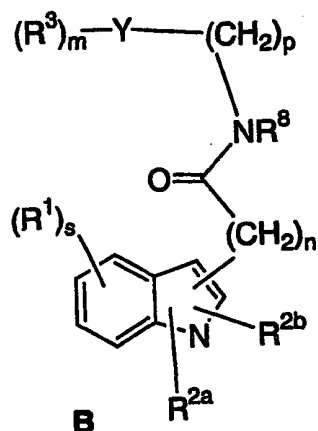
p is 1 to 4;

15 s is 0 to 4;

or a pharmaceutically acceptable salt or stereoisomer thereof.

In another embodiment of this invention, the inhibitors of prenyl-protein transferase are illustrated by the formula B:

20



wherein

25 R¹ is independently selected from

- a) H,

- b) unsubstituted or substituted C₁-C₆ alkyl,
- c) unsubstituted or substituted C₁-C₆ alkoxy,
- d) unsubstituted or substituted aryl,
- e) unsubstituted or substituted perfluoroalkyl,
- 5 f) halo,
- g) OR¹⁰,
- h) R¹⁰C(O)NR¹⁰-,
- i) -C(O)N(R¹⁰)₂,
- j) CN,
- 10 k) R¹⁰C(O)-, or
- l) -N(R¹⁰)₂;

R^{2a} and R^{2b} are independently selected from:

- a) H,
- 15 b) unsubstituted or substituted C₁-C₆ alkyl,
- c) unsubstituted or substituted aryl,
- d) oxo,
- e) unsubstituted or substituted heterocycle, or
- f) unsubstituted or substituted C₁-C₆ alkoxy;

20 wherein the substituted group is substituted with from one to three substituents selected from:

- 1) unsubstituted or substituted C₁-C₆ alkyl,
- 2) unsubstituted or substituted C₁-C₆ alkoxy,
- 3) halo,
- 25 4) unsubstituted or substituted aryl,
- 5) unsubstituted or substituted heterocycle,
- 6) unsubstituted or substituted perfluoroalkyl,
- 7) -OR¹⁰,
- 8) R¹⁰C(O)NR¹⁰-,
- 30 9) -C(O)N(R¹⁰)₂, or
- 10) -N(R¹⁰)₂;

R^{2a} and R^{2b} may optionally be joined in a ring;

R³ is independently selected from:

- a) H,
- b) unsubstituted or substituted C₁-C₆ alkyl,
- c) halo,
- 5 d) -OR¹⁰,
- e) R¹⁰C(O)NR¹⁰-,
- f) -C(O)NR¹⁰,
- g) R¹⁰C(O)-, or
- h) -N(R¹⁰)₂;

10

R⁸ is selected from:

- a) unsubstituted or substituted C₁-C₆ alkyl,
- b) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
- c) unsubstituted or substituted aryl,
- 15 d) unsubstituted or substituted C₂-C₈ alkenyl, or
- e) unsubstituted or substituted C₂-C₈ alkynyl;

wherein the substituted group is selected from:

- 1) unsubstituted or substituted C₁-C₆ alkyl,
- 2) unsubstituted or substituted C₂-C₈ alkenyl,
- 3) unsubstituted or substituted C₂-C₈ alkynyl,
- 20 4) unsubstituted or substituted C₁-C₆ alkoxy,
- 5) CN,
- 6) unsubstituted or substituted aryl, or
- 7) unsubstituted or substituted heterocycle;

25

R¹⁰ is selected from:

- a) H,
- b) unsubstituted or substituted C₁-C₆ alkyl,
- c) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
- 30 d) unsubstituted or substituted aryl,
- e) unsubstituted or substituted heterocycle,
- f) unsubstituted or substituted aralkyl, or
- g) unsubstituted or substituted heterocyclalkyl;

R¹¹ is selected from

- a) unsubstituted or substituted C₁-C₆ alkyl,
- b) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
- c) unsubstituted or substituted aryl,
- 5 d) unsubstituted or substituted heterocycle,
- e) unsubstituted or substituted aralkyl, or
- f) unsubstituted or substituted heterocyclalkyl;

Y is selected from:

- 10 a) heterocycle, which is selected from pyridinyl, furanyl, pyrazolyl, pyrimidinyl, pyrazinyl, imidazolyl, oxazolyl, thiazolyl, triazolyl, tetrazolyl or thiofuranyl;
- b) C₃-C₁₀ cycloalkyl,
- c) C₂-C₈ alkenyl,
- 15 d) C₂-C₈ alkynyl,
- e) C₁-C₆ alkoxy,
- f) CN,
- g) C(O),
- h) C(O)OR¹⁰,
- 20 i) -OR¹⁰,
- j) -N(R¹⁰)₂, or
- k) C(=NH)NHR¹⁰;

m is 0 to 4;

25 n is 0 or 1;

p is 1 to 4;

s is 0 to 4;

or a pharmaceutically acceptable salt or stereoisomer thereof.

30

Examples of the compounds of the instant invention are:

N-isopropyl-2-(1-methyl-2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide;

N-isopropyl-2-(1-methyl-2-o-tolyl-1H-indol-3-yl)-N-pyridin-4-ylmethyl-acetamide;

N-isopropyl-2-(7-methyl-2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide;

- 2-[1-(2-bromobenzyl)-1H-indol-3-yl]-N-isopropyl-N-(pyridin-4-ylmethyl)acetamide;
 N-isopropyl-2-(2-phenyl-1H-indol-1-yl)-N-(pyridin-4-ylmethyl)acetamide;
 N-isopropyl-2-(2-phenyl-1H-pyrrolo[2,3-b]pyridin-3-yl)-N-pyridin-4-ylmethyl-
 acetamide;
- 5 N-benzyl-N-isopropyl-2-(2-phenyl-1H-indol-3-yl)acetamide;
 N-ethyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide;
 2-[1-(4-bromobenzyl)-1H-indol-3-yl]-N-isopropyl-N-(pyridin-4-ylmethyl)acetamide;
 2-[1-(3-bromobenzyl)-1H-indol-3-yl]-N-isopropyl-N-(pyridin-4-ylmethyl)acetamide;
 2-(2-phenyl-1H-indol-3-yl)-N,N-bis(pyridin-3-ylmethyl)acetamide;
- 10 2-(1-benzyl-1H-indol-3-yl)-N-isopropyl-N-(pyridin-4-ylmethyl)acetamide;
 2-[2-(4-bromophenyl)-1H-indol-3-yl]-N-isopropyl-N-(pyridin-4-ylmethyl)acetamide;
 2-(1-ethyl-2-phenyl-1H-indol-3-yl)-N-isopropyl-N-(pyridin-4-ylmethyl)acetamide;
 N-isopropyl-2-(2-phenyl-1-propyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide;
 2-(1-benzyl-2-oxo-2,3-dihydro-1H-indol-3-yl)-N-isopropyl-N-(pyridin-4-ylmethyl)
- 15 acetamide;
 1-benzyl-4-{[(1-benzyl-2-oxo-2,3-dihydro-1H-indol-3-yl)acetyl] (isopropyl)amino]
 methyl}pyridinium;
 N-cyclobutyl-2-(2-phenyl-1H-indol-3-yl)-N-pyridin-3-ylmethyl-acetamide;
 N-cyclopropyl-2-(2-phenyl-1H-indol-3-yl)-N-pyridin-3-ylmethyl-acetamide;
- 20 2-[2-(2-chloro-phenyl)-1H-indol-3-yl]-N-isopropyl-N-pyridin-4-ylmethyl-acetamide;
 N,N-diallyl-2-(2-phenyl-1H-indol-3-yl)-acetamide;
 N-isopropyl-2-(2-phenyl-1H-indol-3-yl)-N-pyridin-3-ylmethyl-acetamide;
 (S)-N-sec-butyl-2-(2-phenyl-1H-indol-3-yl)-N-pyridin-4-ylmethyl-acetamide;
 N-furan-3-ylmethyl-N-isopropyl-2-(2-phenyl-1H-indol-3-yl)-acetamide;
- 25 N-(2-cyano-ethyl)-N-cyclopropyl-2-(2-phenyl-1H-indol-3-yl)-acetamide;
 N-cyclobutyl-2-(2-phenyl-1H-indol-3-yl)-N-pyridin-4-ylmethyl-acetamide;
 N-cyclopropyl-2-(2-phenyl-1H-indol-3-yl)-N-pyridin-4-ylmethyl-acetamide;
 2-[2-(3-chloro-phenyl)-1H-indol-3-yl]-N-isopropyl-N-pyridin-4-ylmethyl-acetamide;
 N-(2-cyano-ethyl)-N-cyclopropyl-2-(1-methyl-2-phenyl-1H-indol-3-yl)-acetamide;
- 30 N-isopropyl-2-(2-phenyl-1H-indol-3-yl)-N-pyrimidin-5-ylmethyl-acetamide;
 N-cyclopropyl-N-(2-methyl-2H-pyrazol-3-ylmethyl)-2-(2-phenyl-1H-indol-3-yl)-
 acetamide;
 (+)-N-isopropyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)propanamide;
 N-(2-furylmethyl)-N-isopropyl-2-(2-phenyl-1H-indol-3-yl)acetamide;

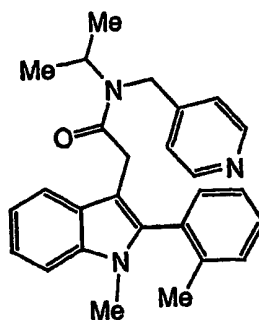
- N-isopropyl-N-[(1-methyl-1H-pyrazol-5-yl)methyl]-2-(2-phenyl-1H-indol-3-yl)acetamide;
- N-isobutyl-N-methyl-2-(2-phenyl-1H-indol-3-yl)acetamide;
- N-cyclopropyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyrazin-2-ylmethyl)acetamide;
- 5 N-cyclopropyl-N-(3-furylmethyl)-2-(2-phenyl-1H-indol-3-yl)acetamide;
- N-(2-cyanoethyl)-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-3-ylmethyl)acetamide;
- N-isopropyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyrazin-2-ylmethyl)acetamide;
- N-isopropyl-N-[(1-methyl-1H-pyrazol-4-yl)methyl]-2-(2-phenyl-1H-indol-3-yl)acetamide;
- 10 N-isopropyl-N-[(1-methyl-1H-pyrazol-3-yl)methyl]-2-(2-phenyl-1H-indol-3-yl)acetamide;
- N-isopropyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-2-ylmethyl)acetamide;
- N-(2-cyanoethyl)-N-cyclopropyl-2-[2-(2-methylphenyl)-1H-indol-3-yl]acetamide;
- N-isopentyl-N-methyl-2-(2-phenyl-1H-indol-3-yl)acetamide;
- 15 N-(2-cyanoethyl)-N-isopropyl-2-(2-phenyl-1H-indol-3-yl)acetamide;
- N-cyclopropyl-N-[(1-methyl-1H-pyrazol-4-yl)methyl]-2-(2-phenyl-1H-indol-3-yl)acetamide;
- N-cyclopropyl-N-[(1-methyl-1H-pyrazol-3-yl)methyl]-2-(2-phenyl-1H-indol-3-yl)acetamide;
- 20 N-cyclobutyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-2-ylmethyl)acetamide;
- N-cyclopropyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-2-ylmethyl)acetamide;
- N-cyclopropyl-N-(2-furylmethyl)-2-(2-phenyl-1H-indol-3-yl)acetamide;
- N-isopropyl-2-[2-(2-methoxyphenyl)-1H-indol-3-yl]-N-(pyridin-4-ylmethyl)acetamide;
- 25 N-isobutyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-2-ylmethyl)acetamide;
- 2-[2-(2-chlorophenyl)-1H-indol-3-yl]-N-(2-cyanoethyl)-N-cyclopropylacetamide;
- N-(tert-butyl)-N-(2-cyanoethyl)-2-(2-phenyl-1H-indol-3-yl)acetamide;
- N-isobutyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-3-ylmethyl)acetamide;
- methyl N-cyclopropyl-N-[(2-phenyl-1H-indol-3-yl)acetyl]-beta-alaninate;
- 30 N-isopropyl-2-(6-methyl-2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide;
- N-isopropyl-2-(4-methyl-2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide;
- N-isobutyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide;
- N-cyclopentyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide;
- N-[(1R)-1-methylpropyl]-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)
- 35 acetamide;

- N,N-bis(2-methoxyethyl)-2-(2-phenyl-1H-indol-3-yl)acetamide;
 N-isopropyl-2-(5-methyl-2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide;
 N-(2-cyanoethyl)-N-cyclopropyl-2-[2-(2-methoxyphenyl)-1H-indol-3-yl]acetamide;
 N-cyclopentyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-2-ylmethyl)acetamide;
 5 N-cyclopentyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-3-ylmethyl)acetamide;
 N-isopropyl-N-phenyl-2-(2-phenyl-1H-indol-3-yl)acetamide;
 (2-phenyl-1H-indol-3-yl)acetyl N-[3-(dimethylamino)propyl]-N'-ethylimidocarbamate;
 N-cyclopropyl-N-[(2-phenyl-1H-indol-3-yl)acetyl]-beta-alanine;
 10 N-Isopropyl-2-(2-pyridin-3-yl-1H-indol-3-yl)-N-pyridin-4-ylmethyl-acetamide;
 N-Isopropyl-2-(2-pyridin-4-yl-1H-indol-3-yl)-N-pyridin-3-ylmethyl-acetamide;
 N-Benzyl-N-isopropyl-2-(2-pyridin-4-yl-1H-indol-3-yl)-acetamide;

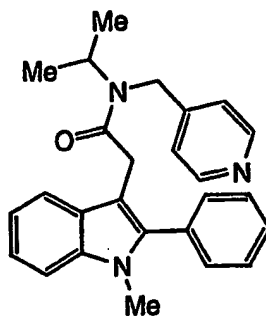
or a pharmaceutically acceptable salt or stereoisomer thereof.

15

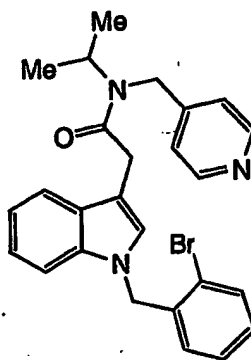
Specific examples of the compounds of the instant invention are illustrated by



- 20 N-isopropyl-2-(1-methyl-2-o-tolyl-1H-indol-3-yl)-N-pyridin-4-ylmethyl-acetamide;

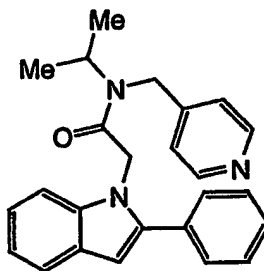


N-isopropyl-2-(1-methyl-2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide;

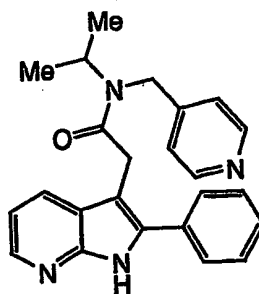


5.

2-[1-(2-bromobenzyl)-1H-indol-3-yl]-N-isopropyl-N-(pyridin-4-ylmethyl)acetamide;

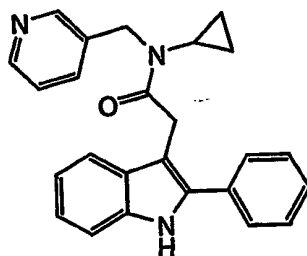


10 N-isopropyl-2-(2-phenyl-1H-indol-1-yl)-N-(pyridin-4-ylmethyl)acetamide;

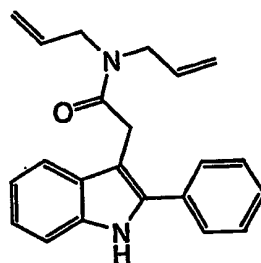


N-isopropyl-2-(2-phenyl-1H-pyrrolo[2,3-b]pyridin-3-yl)-N-pyridin-4-ylmethyl-acetamide;

5

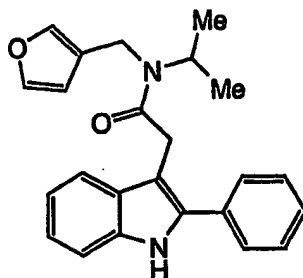


N-cyclopropyl-2-(2-phenyl-1H-indol-3-yl)-N-pyridin-3-ylmethyl-acetamide;

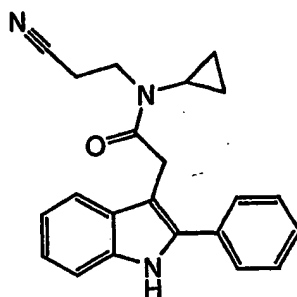


10

N,N-diallyl-2-(2-phenyl-1H-indol-3-yl)-acetamide;



N-furan-3-ylmethyl-N-isopropyl-2-(2-phenyl-1H-indol-3-yl)-acetamide;



5

N-(2-cyano-ethyl)-N-cyclopropyl-2-(2-phenyl-1H-indol-3-yl)-acetamide;

or a pharmaceutically acceptable salt or stereoisomer thereof.

10 The compounds of the present invention may have asymmetric centers, chiral axes and chiral planes, and occur as racemates, racemic mixtures, and as individual diastereomers, with all possible isomers, including optical isomers, being included in the present invention. (See E.L. Eliel and S.H. Wilen *Stereochemistry of Carbon Compounds* (John Wiley and Sons, New York 1994),
15 in particular pages 1119-1190) When any variable (e.g. aryl, heterocycle, R^{1a}, R³ etc.) occurs more than one time in any constituent, its definition on each occurrence is independent at every other occurrence. Also, combinations of substituents/or variables are permissible only if such combinations result in stable compounds.

20 As used herein, "alkyl" is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups having 1 to 6 carbon atoms, unless otherwise indicated; "alkoxy" represents an alkyl group having 1 to 6 carbon atoms, unless otherwise indicated, attached through an oxygen bridge. "Halogen"

or "halo" as used herein means fluoro, chloro, bromo and iodo.

As used herein, "cycloalkyl" is intended to include non-aromatic hydrocarbon groups having having from 3 to 10 carbon atoms, unless otherwise specified. Examples of such cycloalkyl groups includes, but are not limited to, cyclopropyl, cyclobutyl, cyclohexyl, cycloheptyl, cyclooctyl, admantyl and the like. 5 Optionally, a carbon atom in the cycloalkyl may be replaced with a heteroatom, such as O, N or S.

If no number of carbon atoms is specified, the term "alkenyl" refers to a non-aromatic hydrocarbon, straight, branched or cyclic, containing from 2 to 10 carbon atoms, unless otherwise indicated, and at least one carbon to carbon double bond. Preferably one carbon to carbon double bond is present, and up to four non-aromatic carbon-carbon double bonds may be present. Thus, "C₂-C₈ alkenyl" means an alkenyl radical having from 2 to 8 carbon atoms. Examples of such alkenyl groups include, but are not limited to, ethenyl, propenyl, butenyl and cyclohexenyl. 10 As described above with respect to alkyl, the straight, branched or cyclic portion of the alkenyl group may contain double bonds and may be substituted if a substituted alkenyl group is indicated.

The term "alkynyl" refers to a hydrocarbon radical straight, branched or cyclic, containing from 2 to 10 carbon atoms, unless otherwise indicated, and at least one carbon to carbon triple bond. Up to three carbon-carbon triple bonds may be present. Thus, "C₂-C₈ alkynyl" means an alkynyl radical having from 2 to 8 carbon atoms. Examples of such alkynyl groups include, but are not limited to, ethynyl, propynyl and butynyl. As described above with respect to alkyl, the straight, branched or cyclic portion of the alkynyl group may contain triple bonds and may be substituted if a substituted alkynyl group is indicated. 20 25

As used herein, "aryl" is intended to mean any stable monocyclic or bicyclic carbon ring of up to 7 members in each ring, wherein at least one ring is aromatic. Examples of such aryl elements include, but are not limited to, phenyl, naphthyl, tetrahydronaphthyl, indanyl, biphenyl, phenanthryl, anthryl, acenaphthyl and the like. 30

As used herein, "aralkyl" is intended to mean an aryl moiety, as defined above, attached through a C₁-C₆ alkyl linker, where alkyl is defined above. Examples of aralkyls include, but are not limited to, benzyl, naphthylmethyl, phenylbutyl and the like.

35 The term heterocycle or heterocyclic, as used herein, represents

a stable 5- to 7-membered monocyclic or stable 8- to 11-membered bicyclic heterocyclic ring which is either saturated or unsaturated, and which consists of carbon atoms and from one to four heteroatoms selected from the group consisting of N, O, and S, and including any bicyclic group in which any of the above-defined
5 heterocyclic rings is fused to a benzene ring. The heterocyclic ring may be attached at any heteroatom or carbon atom which results in the creation of a stable structure. The term heterocycle or heterocyclic includes heteroaryl moieties. Examples of such heterocyclic elements include, but are not limited to, azepinyl, benzimidazolyl, benzisoxazolyl, benzofurazanyl, benzopyranyl, benzothiopyranyl, benzofuryl, benzo-
10 thiazolyl, benzothienyl, benzoxazolyl, chromanyl, cinnolinyl, dihydrobenzofuryl, dihydrobenzothienyl, dihydrobenzothiopyranyl, dihydrobenzothiopyranyl sulfone, 1,3-dioxolanyl, furyl, imidazolidinyl, imidazolyl, indolinyl, indolyl, isochromanyl, isoindolinyl, isoquinolinyl, isothiazolidinyl, isothiazolyl, isothiazolidinyl, morpholinyl, naphthyridinyl, oxadiazolyl, 2-oxoazepinyl, oxazolyl, 2-oxopiperazinyl, 2-
15 oxopiperdinyl, 2-oxopyrrolidinyl, piperidyl, piperazinyl, pyridyl, 2-pyridinonyl pyrazinyl, pyrazolidinyl, pyrazolyl, pyridazinyl, pyrimidinyl, pyrrolidinyl, pyrrolyl, quinazolinyl, quinolinyl, quinoxalinyl, tetrahydrofuryl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, thiamorpholinyl, thiamorpholinyl sulfoxide, thiazolyl, thiazolinyl, thienofuryl, thienothienyl, thienyl and triazolyl.

20 As used herein, "heteroaryl" is intended to mean any stable monocyclic or bicyclic carbon ring of up to 7 members in each ring, wherein at least one ring is aromatic and wherein from one to four carbon atoms are replaced by heteroatoms selected from the group consisting of N, O, and S. Examples of such heterocyclic elements include, but are not limited to, benzimidazolyl, benzisoxazolyl,
25 benzofurazanyl, benzopyranyl, benzothiopyranyl, benzofuryl, benzothiazolyl, benzothienyl, benzoxazolyl, chromanyl, cinnolinyl, dihydrobenzofuryl, dihydrobenzothienyl, dihydrobenzothiopyranyl, dihydrobenzothiopyranyl sulfone, furyl, imidazolyl, indolinyl, indolyl, isochromanyl, isoindolinyl, isoquinolinyl, isothiazolyl, naphthyridinyl, oxadiazolyl, pyridyl, pyrazinyl, pyrazolyl, pyridazinyl,
30 pyrimidinyl, pyrrolyl, quinazolinyl, quinolinyl, quinoxalinyl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, thiazolyl, thienofuryl, thienothienyl, thienyl and triazolyl.

As used herein, "heterocyclalkyl" is intended to mean a heterocyclic moiety, as defined above, attached through a C₁-C₆ alkyl linker, where alkyl is defined above. Examples of heterocyclalkyls include, but are not limited to,
35 2-pyridylmethyl, 2-imidazolylethyl, 2-quinolinylmethyl, 2-imidazolylmethyl,

1-piperazineethyl, and the like.

As used herein, the terms "substituted alkyl", "substituted alkenyl", "substituted alkynyl" and "substituted alkoxy", unless otherwise defined, are intended to include the branch or straight-chain alkyl group of the specified number of carbon atoms, wherein the carbon atoms may be substituted with F, Cl, Br, I, CF₃, OCF₃,
 5 CN, N₃, NO₂, NH₂, N(C₁-C₆ alkyl)₂, oxo, OH, -O(C₁-C₆ alkyl), -C(O)H, S(O)₀₋₂, (C₁-C₆ alkyl)S(O)₀₋₂, C₂-C₆ alkenyl, C₂-C₆ alkynyl, -(C₁-C₆ alkyl)S(O)₀₋₂ (C₁-C₆ alkyl), C₃-C₂₀ cycloalkyl, -C(O)NH₂, HC(O)NH-, (C₁-C₆ alkyl)C(O)NH-, H₂NC(O)NH-, (C₁-C₆ alkyl)C(O)-, -O(C₁-C₆ alkyl)CF₃, (C₁-C₆ alkyl)OC(O)-,
 10 (C₁-C₆ alkyl)O(C₁-C₆ alkyl)-, (C₁-C₆ alkyl)C(O)₂(C₁-C₆ alkyl)-, (C₁-C₆ alkyl)OC(O)NH-, aryl, heterocycle, aralkyl, heterocyclalkyl, halo-aryl, halo-aralkyl, halo-heterocycle, halo-heterocyclalkyl, cyano-aryl, cyano-aralkyl, cyano-heterocycle and cyano-heterocyclalkyl.

As used herein, the terms "substituted aryl", "substituted heterocycle", "substituted heteroaryl", "substituted cycloalkyl", "substituted benzyl", "substituted aralkyl" and "substituted heterocyclalkyl", unless otherwise defined, are intended to include the cyclic group containing from 1 to 3 substituents in addition to the point of attachment to the rest of the compound. Such substituents are preferably selected from the group which includes but is not limited to F, Cl, Br, I, CF₃, OCF₃, NH₂,
 15 N(C₁-C₆ alkyl)₂, NO₂, CN, N₃, C₁-C₂₀ alkyl, C₃-C₂₀ cycloalkyl, -OH, -O(C₁-C₆ alkyl), S(O)₀₋₂, (C₁-C₆ alkyl)S(O)₀₋₂, (C₁-C₆ alkyl)S(O)₀₋₂(C₁-C₆ alkyl)-, -C(O)NH₂, HC(O)NH-, (C₁-C₆ alkyl)C(O)NH-, H₂NC(O)NH-, (C₁-C₆ alkyl)C(O)-, (C₁-C₆ alkyl)OC(O)-, (C₁-C₆ alkyl)O(C₁-C₆ alkyl)-, (C₁-C₆)C(O)₂(C₁-C₆ alkyl)-, (C₁-C₆ alkyl)OC(O)NH-, aryl, aralkyl, heterocycle, heterocyclalkyl, halo-aryl,
 20 halo-aralkyl, haloheterocycle, haloheterocyclalkyl, cyano-aryl, cyano-aralkyl, cyano-heterocycle and cyano-heterocyclalkyl.

Lines drawn into the ring systems from substituents (such as from R¹, R^{2a}, R³ etc.) indicate that the indicated bond may be attached to any of the substitutable ring carbon or nitrogen atoms.

30 Preferably, R¹ is independently selected from: hydrogen, OR¹⁰, -N(R¹⁰)₂, halo or unsubstituted or substituted C₁-C₆ alkyl.

Preferably, R^{1a} and R^{1b} are independently selected from: hydrogen, or unsubstituted or substituted C₁-C₆ alkyl.

35 Preferably, R^{2a} and R^{2b} are independently selected from: hydrogen, unsubstituted or substituted C₁-C₆ alkyl, unsubstituted or substituted aryl, oxo or

unsubstituted or substituted C₁-C₆ alkoxy. Most preferably, R^{2a} is unsubstituted or substituted aryl.

Preferably, R³ is independently selected from: hydrogen, unsubstituted or substituted C₁-C₆ alkyl, unsubstituted or substituted aryl and OR¹⁰.

5 Preferably, R⁸ is selected from hydrogen, unsubstituted or substituted C₁-C₆ alkyl, unsubstituted or substituted C₂-8 alkenyl, or unsubstituted or substituted C₃-10 cycloalkyl.

10 Preferably, Y is selected from heterocycle, C₃-10 cycloalkyl, C(O), CN, or -OR¹⁰. If Y is a heterocycle, preferably, the heterocycle is selected from pyridinyl, furanyl, pyrazolyl, pyrimidinyl, pyrazinyl, imidazolyl, oxazolyl, thiazolyl, triazolyl, tetrazolyl or thiofuranyl. If Y is a heterocycle, most preferably, the heterocycle is selected from pyridinyl, furanyl, pyrazolyl, pyrimidinyl, or pyrazinyl.

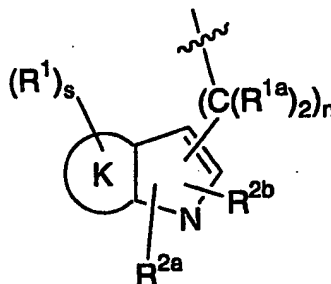
Preferably, m is 0 or 1.

Preferably, n is 0, 1, or 2. Most preferably, n is 1.

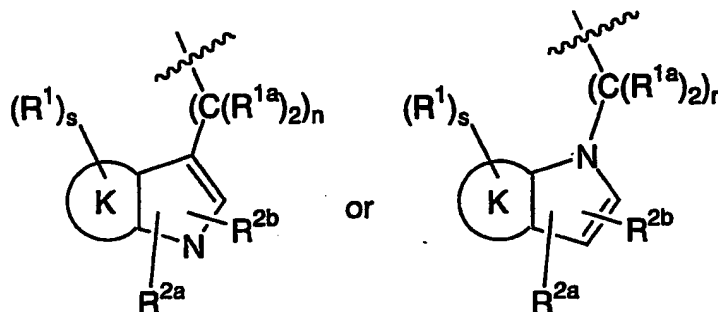
15 Preferably p is 0, 1, or 2.

Preferably s is 0 or 1.

Preferably, the moiety

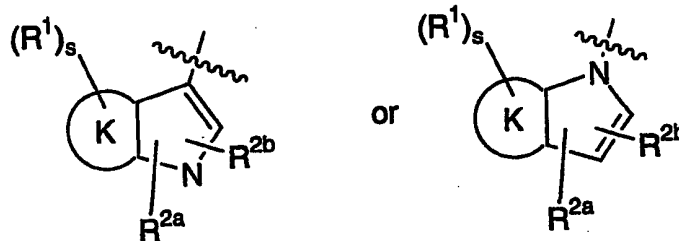


is selected from

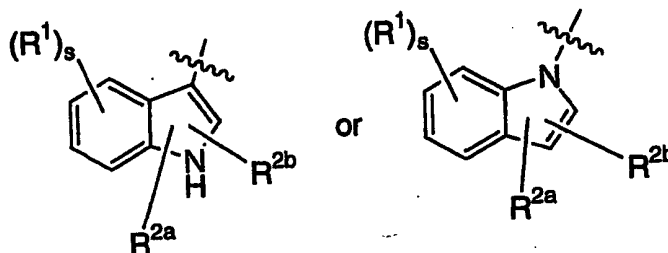


20

More preferably,



is selected from



5

It is intended that the definition of any substituent or variable (e.g., R^{1a} , R^3 , n , etc.) at a particular location in a molecule be independent of its definitions elsewhere in that molecule. Thus, $-N(R^{10})_2$ represents $-NHH$, $-NHCH_3$, $-NHC_2H_5$, etc. It is understood that substituents and substitution patterns on the compounds of the instant invention can be selected by one of ordinary skill in the art to provide compounds that are chemically stable and that can be readily synthesized by techniques known in the art, as well as those methods set forth below, from readily available starting materials.

The pharmaceutically acceptable salts of the compounds of this invention include the conventional non-toxic salts of the compounds of this invention as formed, e.g., from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like: and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pantoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxy-benzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, trifluoroacetic and the like.

The pharmaceutically acceptable salts of the compounds of this invention can be synthesized from the compounds of this invention which contain a basic moiety by conventional chemical methods. Generally, the salts are prepared either by ion exchange chromatography or by reacting the free base with stoichiometric amounts or with an excess of the desired salt-forming inorganic or organic acid in a suitable solvent or various combinations of solvents.

Abbreviations which may be used in the description of the chemistry and in the Examples that follow include:

10	Ac ₂ O	Acetic anhydride;
	AIBN	2,2'-Azobisisobutyronitrile;
	BINAP	2,2'-Bis(diphenylphosphino)-1,1' binaphthyl;
	Bn	Benzyl;
	BOC/Boc	t-Butoxycarbonyl;
15	CBz	Carbobenzyloxy;
	DBAD	Di- <i>tert</i> -butyl azodicarboxylate;
	DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene;
	DCE	1,2-Dichloroethane;
	DIEA	<i>N,N</i> -Diisopropylethylamine;
20	DMAP	4-Dimethylaminopyridine;
	DME	1,2-Dimethoxyethane;
	DMF	<i>N,N</i> -Dimethylformamide;
	DMSO	Methyl sulfoxide;
	DPPA	Diphenylphosphoryl azide;
25	DTT	Dithiothreitol;
	EDC	1-(3-Dimethylaminopropyl)-3-ethyl-carbodiimide-hydrochloride;
	EDTA	Ethylenediaminetetraacetic acid;
	Et ₂	Diethyl ether;
	Et ₃ N	Triethylamine;
30	EtOAc	Ethyl acetate;
	EtOH	Ethanol;
	FAB	Fast atom bombardment;
	HEPES	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid;
	HOAc	Acetic acid;
35	HOBT	1-Hydroxybenzotriazole hydrate;

	HOObt	3-Hydroxy-1,2,2-benzotriazin-4(3H)-one;
	HPLC	High-performance liquid chromatography;
	KOtBu	Potassium t-butoxide;
	LAH	Lithium aluminum hydride;
5	MCPBA	<i>m</i> -Chloroperoxybenzoic acid;
	Me	Methyl;
	MeOH	Methanol;
	Ms	Methanesulfonyl;
	MsCl	Methanesulfonyl chloride;
10	<i>n</i> -Bu	<i>n</i> -butyl;
	<i>n</i> -Bu ₃ P	Tri- <i>n</i> -butylphosphine;
	NaHMDS	Sodium bis(trimethylsilyl)amide;
	NBS	<i>N</i> -Bromosuccinimide;
	Pd(PPh ₃) ₄	Palladium tetrakis(triphenylphosphine);
15	Pd ₂ (dba) ₂	Tris(dibenzylideneacetone)dipalladium (0)
	Ph	phenyl;
	PMSF	<i>p</i> -Toluenesulfonyl chloride;
	Py or pyr	Pyridine;
	PYBOP	Benzotriazole-1-yl-oxy-trispyrrolidinophosphonium
20	(or PyBOP)	hexafluorophosphate;
	<i>t</i> -Bu	<i>tert</i> -Butyl;
	TBAF	Tetrabutylammoniumfluoride;
	RPLC	Reverse Phase Liquid Chromatography;
	RT	Room Temperature;
25	TBSCl	<i>tert</i> -Butyldimethylsilyl chloride;
	TFA	Trifluoroacetic acid;
	THF	Tetrahydrofuran;
	TIPS	Triisopropylsilyl;
	TMS	Tetramethylsilane; and
30	Tr	Trityl.

These reactions may be employed in a linear sequence to provide the compounds of the invention or they may be used to synthesize fragments which are subsequently joined by the alkylation reactions described in the Schemes.

Synopsis of Schemes 1-6:

In the schemes below, the substituents and variables illustrated (such as n, R^{2a}, R⁸, Y, etc.) are defined in Formula I. Substituent R depicts the possible one to three substituents available when substituents R^{2a} or R^{2b}, of Formula I, are substituted.

5 The requisite intermediates are in some cases commercially available, or can be prepared according to literature procedures, for the most part. In Scheme 1, for example, the synthesis of 2-phenylindole-3-acetamides is described. To a solution of aldehyde 1 and amine 2 in methanol at 0°C is added sodium borohydride. 10 Reductive amination product 3 is then coupled with 2-phenylindole-3-acetic acid (4) to give the target amide 5. N-alkylation of amide 5 can also be effected by treatment with potassium *tert*-butoxide and an alkyl iodide, providing compound 6.

Preparation of indole-3-acetamides with various substituents attached at the 2-position is illustrated in Scheme 2. Indole-3-acetonitrile (7) is brominated to 15 give intermediate 8. Subsequent Suzuki coupling provides 2-substituted compound 9. Hydrolysis of the cyano group affords acid 10, which is then coupled with amine 3 to provide compound 11.

Scheme 3 depicts the preparation of indoles substituted at the 4, 5, 6, and/or 7 positions. Acyl substituted acid 12 is coupled to amine 3 under standard 20 conditions to provide amide 13. Formation of hydrazone 15 is followed by Fischer indolization to give indole 16. Using acids of type 12 substituted at the 2-alkyl position provides the corresponding alpha-substituted indoleacetamides.

Scheme 4 details the synthesis of N-arylmethyl substituted indoleacetamides. Indole-3-acetic acid (17) is coupled to amine 3 to provide amide 25 18. Subsequent alkylation with bromide 19 affords the N-alkylated product 20.

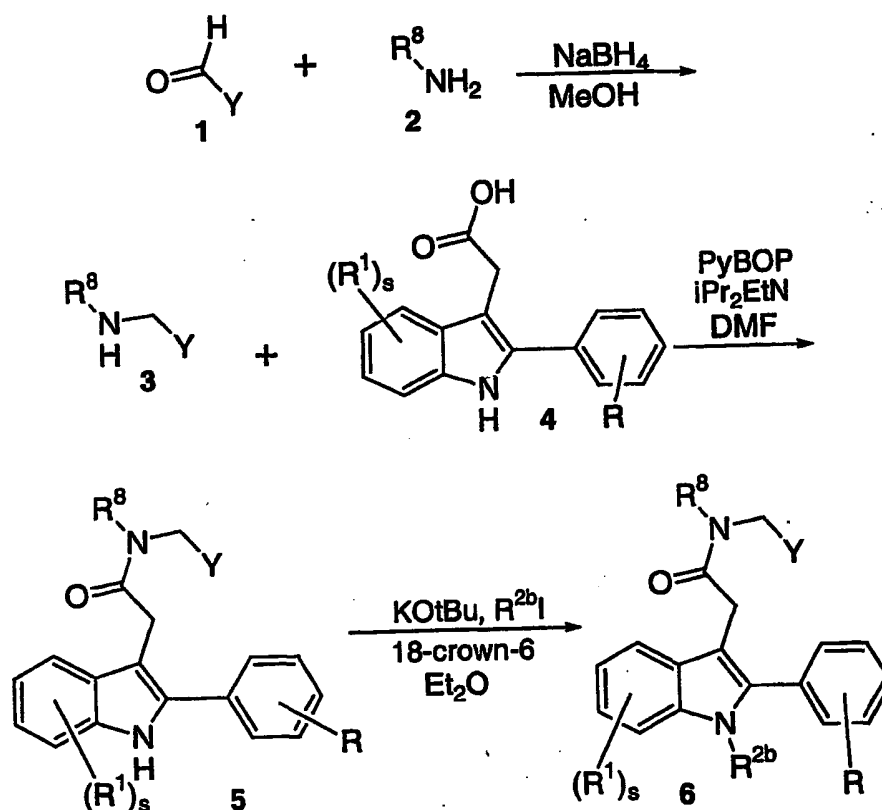
The isomeric indoles 24 can be prepared as described in Scheme 5. Treatment of 2-substituted indole 22 with potassium hydroxide and methyl bromoacetate in wet DMSO effects alkylation and ester cleavage to provide acid 23. Peptide coupling using amine 3 gives compound 24.

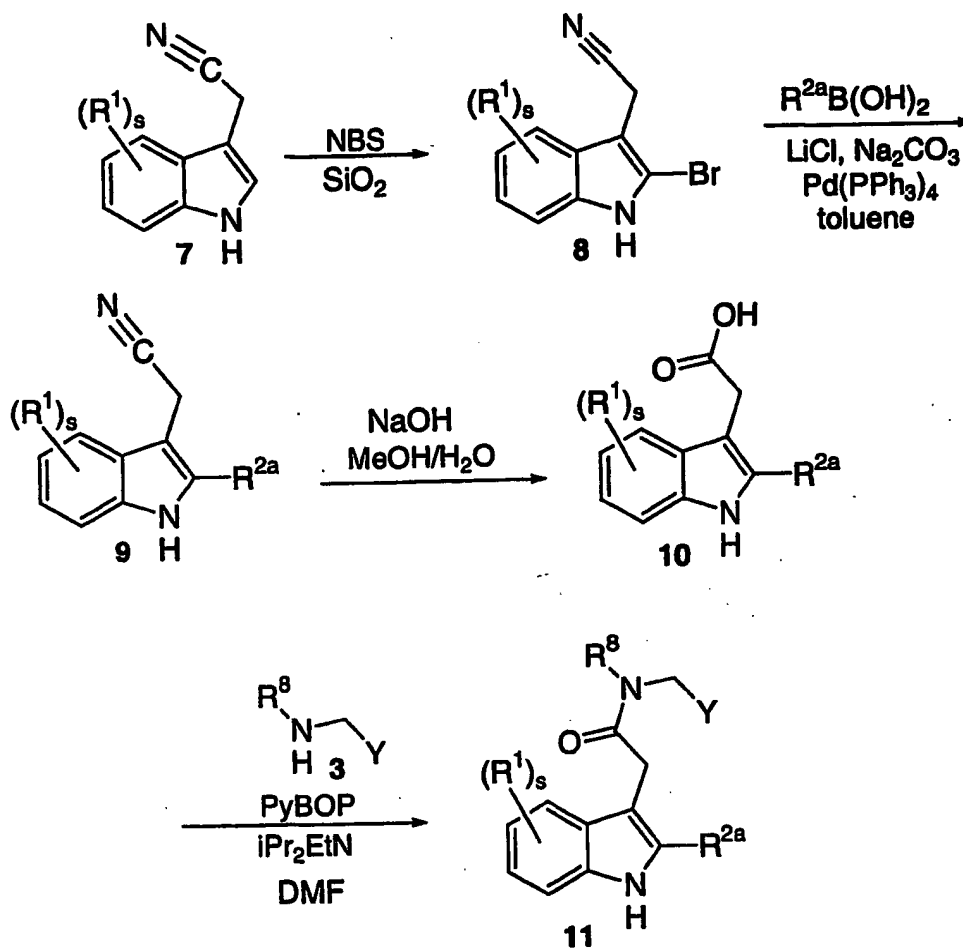
30 The synthesis of 2-substituted-azaindole-3-acetamides is outlined in Scheme 6. Azaindole 27 is prepared from aminopyridine 25 and Weinreb amide 26 using the method of Hands et al. (Hands, D.; Bishop, B.; Cameron, M.; Edwards, J. S.; Cottrell, I. F.; Wright, S. H. B. *Synthesis* 1996, 877-882). A Mannich reaction with paraformaldehyde as the amine component yields azagramine 28. *In situ*

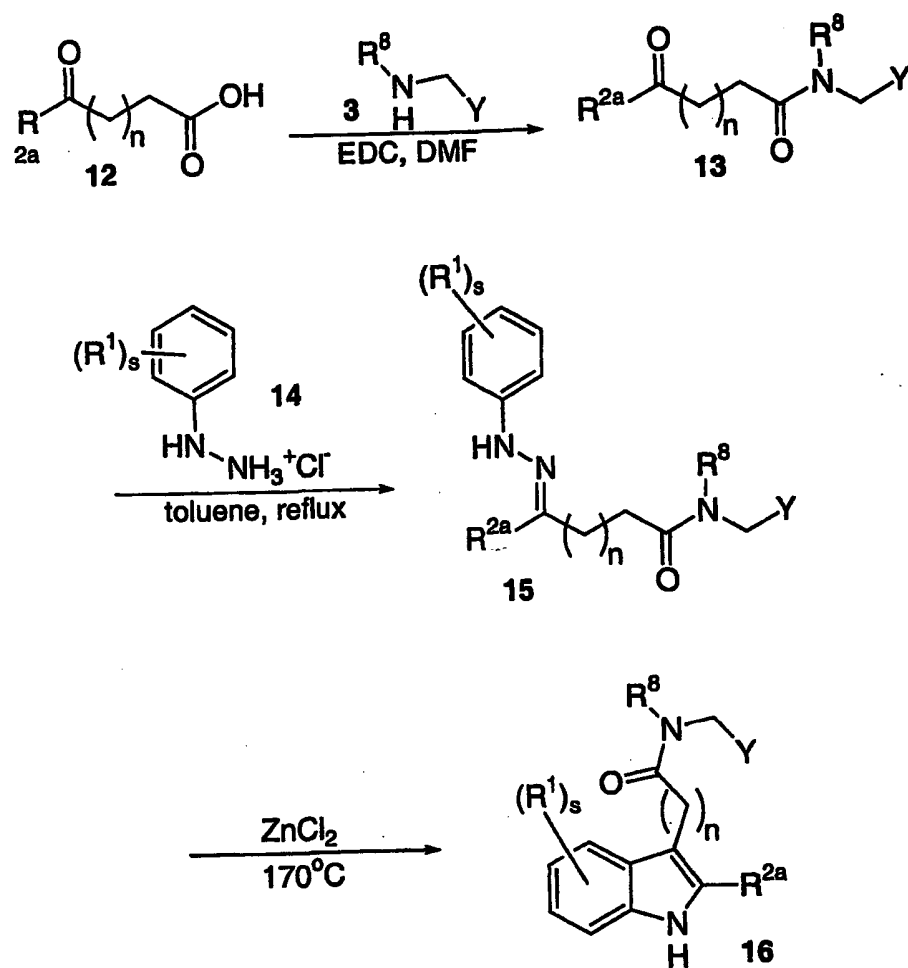
quaternization and cyanide displacement provides indolyl acetonitrile **29**. Basic hydrolysis and peptide coupling as illustrated above then provides acetamide **31**.

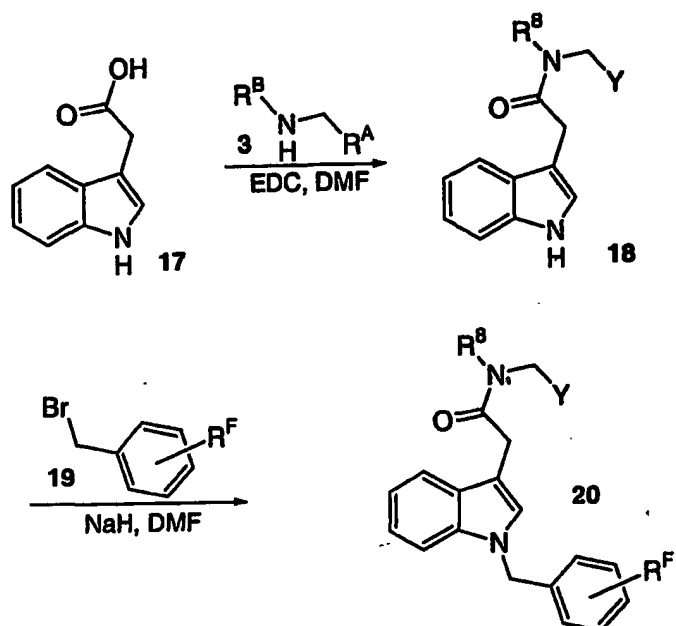
SCHEME 1

5

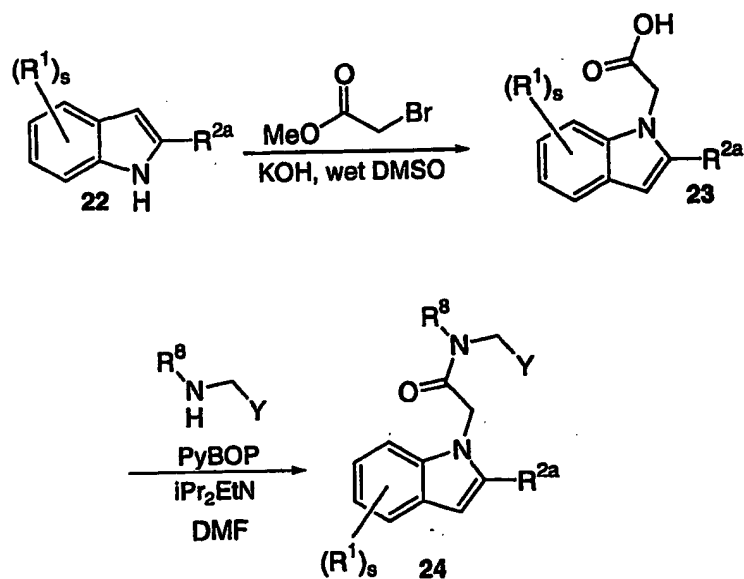


SCHEME 2

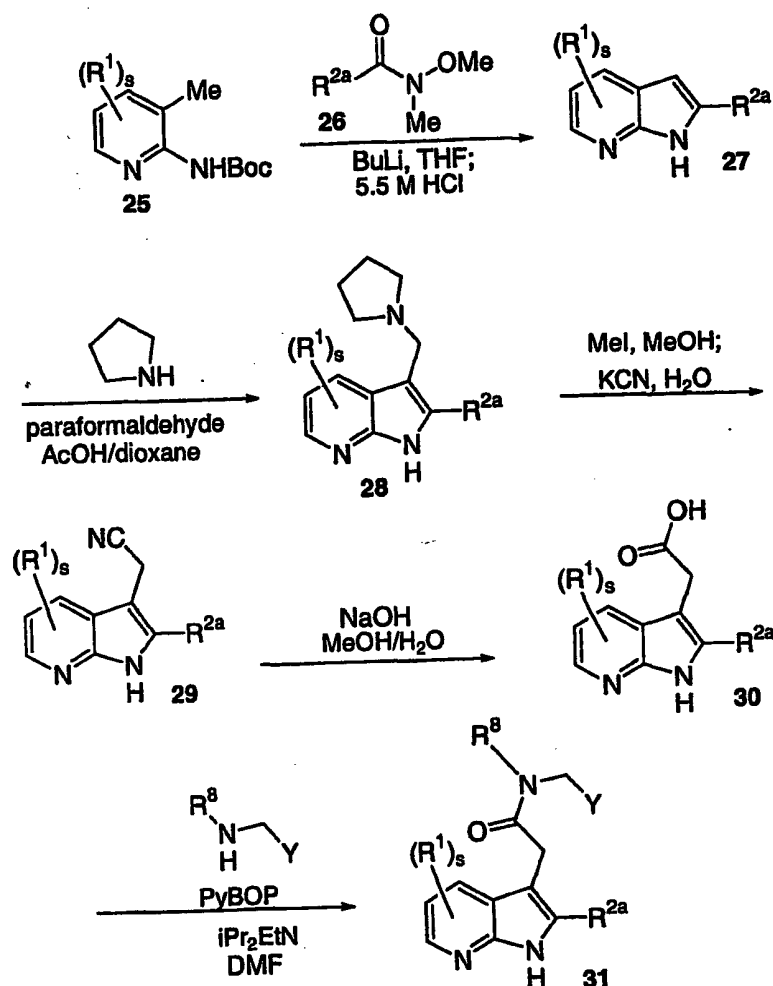
SCHEME 3

SCHEME 4

5

SCHEME 5

SCHEME 6



- 5 In a preferred embodiment of the instant invention the compounds of the invention are selective inhibitors of farnesyl-protein transferase. A compound is considered a selective inhibitor of farnesyl-protein transferase, for example, when its *in vitro* farnesyl-protein transferase inhibitory activity, as assessed by the assay described in Example 67, is at least 100 times greater than the *in vitro* activity of
- 10 the same compound against geranylgeranyl-protein transferase-type I in the assay described in Example 68. Preferably, a selective compound exhibits at least 1000 times greater activity against one of the enzymatic activities when comparing

geranylgeranyl-protein transferase-type I inhibition and farnesyl-protein transferase inhibition.

It is also preferred that the selective inhibitor of farnesyl-protein transferase is further characterized by:

- 5 a) an IC₅₀ (a measure of *in vitro* inhibitory activity) for inhibition of the prenylation of newly synthesized K-Ras protein more than about 100-fold higher than the EC₅₀ for the inhibition of the farnesylation of hDJ protein. When measuring such IC₅₀s and EC₅₀s the assays described in Example 72 may be utilized.

10 It is also preferred that the selective inhibitor of farnesyl-protein transferase is further characterized by:

- b) an IC₅₀ (a measurement of *in vitro* inhibitory activity) for inhibition of K4B-Ras dependent activation of MAP kinases in cells at least 100-fold greater than the EC₅₀ for inhibition of the farnesylation of the protein hDJ in cells.

15 It is also preferred that the selective inhibitor of farnesyl-protein transferase is further characterized by:

- c) an IC₅₀ (a measurement of *in vitro* inhibitory activity) against H-Ras dependent activation of MAP kinases in cells at least 1000 fold lower than the inhibitory activity (IC₅₀) against H-ras-CVLL (SEQ.ID.NO.: 1) dependent activation of MAP kinases in cells.

20 When measuring Ras dependent activation of MAP kinases in cells the assays described in Example 71 may be utilized.

In another preferred embodiment of the instant invention the compounds of the invention are dual inhibitors of farnesyl-protein transferase and geranylgeranyl-protein transferase type I. Such a dual inhibitor may be termed a
25 Class II prenyl-protein transferase inhibitor and will exhibit certain characteristics when assessed in *in vitro* assays, which are dependent on the type of assay employed.

In a SEAP assay, such as described in Example 71, it is preferred that the dual inhibitor compound has an *in vitro* inhibitory activity (IC₅₀) that is less than
30 about 12μM against K4B-Ras dependent activation of MAP kinases in cells.

The Class II prenyl-protein transferase inhibitor may also be characterized by:

- a) an IC₅₀ (a measurement of *in vitro* inhibitory activity) for inhibiting K4B-Ras dependent activation of MAP kinases in cells between 0.1 and 100 times the
35 IC₅₀ for inhibiting the farnesylation of the protein hDJ in cells; and

- b) an IC₅₀ (a measurement of *in vitro* inhibitory activity) for inhibiting K4B-Ras dependent activation of MAP kinases in cells greater than 5-fold lower than the inhibitory activity (IC₅₀) against expression of the SEAP protein in cells transfected with the pCMV-SEAP plasmid that constitutively expresses the SEAP protein.

The Class II prenyl-protein transferase inhibitor may also be characterized by:

- a) an IC₅₀ (a measurement of *in vitro* inhibitory activity) against H-Ras dependent activation of MAP kinases in cells greater than 2 fold lower but less than 20,000 fold lower than the inhibitory activity (IC₅₀) against H-ras-CVLL (SEQ.ID.NO.: 1) dependent activation of MAP kinases in cells; and
- b) an IC₅₀ (a measurement of *in vitro* inhibitory activity) against H-ras-CVLL dependent activation of MAP kinases in cells greater than 5-fold lower than the inhibitory activity (IC₅₀) against expression of the SEAP protein in cells transfected with the pCMV-SEAP plasmid that constitutively expresses the SEAP protein.

The Class II prenyl-protein transferase inhibitor may also be characterized by:

- a) an IC₅₀ (a measurement of *in vitro* inhibitory activity) against H-Ras dependent activation of MAP kinases in cells greater than 10-fold lower but less than 2,500 fold lower than the inhibitory activity (IC₅₀) against H-ras-CVLL (SEQ.ID.NO.: 1) dependent activation of MAP kinases in cells; and
- b) an IC₅₀ (a measurement of *in vitro* inhibitory activity) against H-ras-CVLL dependent activation of MAP kinases in cells greater than 5 fold lower than the inhibitory activity (IC₅₀) against expression of the SEAP protein in cells transfected with the pCMV-SEAP plasmid that constitutively expresses the SEAP protein.

A method for measuring the activity of the inhibitors of prenyl-protein transferase, as well as the instant combination compositions, utilized in the instant methods against Ras dependent activation of MAP kinases in cells is described in Example 71.

In yet another embodiment, a compound of the instant invention may be a more potent inhibitor of geranylgeranyl-protein transferase-type I than it is an inhibitor of farnesyl-protein transferase.

The instant compounds are useful as pharmaceutical agents for mammals, especially for humans. These compounds may be administered to patients for use in the treatment of cancer. Examples of the type of cancer which may be treated with the compounds of this invention include, but are not limited to, colorectal carcinoma, exocrine pancreatic carcinoma, myeloid leukemias and neurological tumors. Such tumors may arise by mutations in the ras genes themselves, mutations in the proteins that can regulate Ras activity (i.e., neurofibromin (NF-1), neu, src, abl, lck, fyn) or by other mechanisms.

The compounds of the instant invention inhibit farnesyl-protein transferase and the farnesylation of the oncogene protein Ras. The instant compounds may also inhibit tumor angiogenesis, thereby affecting the growth of tumors (J. Rak et al. *Cancer Research*, 55:4575-4580 (1995)). Such anti-angiogenesis properties of the instant compounds may also be useful in the treatment of certain forms of vision deficit related to retinal vascularization.

The compounds of this invention are also useful for inhibiting other proliferative diseases, both benign and malignant, wherein Ras proteins are aberrantly activated as a result of oncogenic mutation in other genes (i.e., the Ras gene itself is not activated by mutation to an oncogenic form) with said inhibition being accomplished by the administration of an effective amount of the compounds of the invention to a mammal in need of such treatment. For example, the composition is useful in the treatment of neurofibromatosis, which is a benign proliferative disorder.

The instant compounds may also be useful in the treatment of certain viral infections, in particular in the treatment of hepatitis delta and related viruses (J.S. Glenn et al. *Science*, 256:1331-1333 (1992)).

The compounds of the instant invention are also useful in the prevention of restenosis after percutaneous transluminal coronary angioplasty by inhibiting neointimal formation (C. Indolfi et al. *Nature medicine*, 1:541-545(1995)).

The instant compounds may also be useful in the treatment and prevention of polycystic kidney disease (D.L. Schaffner et al. *American Journal of Pathology*, 142:1051-1060 (1993) and B. Cowley, Jr. et al. *FASEB Journal*, 2:A3160 (1988)).

The instant compounds may also be useful for the treatment of fungal infections.

The instant compounds may also be useful as inhibitors of proliferation of vascular smooth muscle cells and therefore useful in the prevention and therapy of arteriosclerosis and diabetic vascular pathologies.

5 The compounds of the instant invention may also be useful in the prevention and treatment of endometriosis, uterine fibroids, dysfunctional uterine bleeding and endometrial hyperplasia.

10 In such methods of prevention and treatment as described herein, the prenyl-protein transferase inhibitors of the instant invention may also be co-administered with other well known therapeutic agents that are selected for their particular usefulness against the condition that is being treated. For example, the prenyl-protein transferase inhibitor may be useful in further combination with drugs known to suppress the activity of the ovaries and slow the growth of the endometrial tissue. Such drugs include but are not limited to oral contraceptives, progestins, danazol and GnRH (gonadotropin-releasing hormone) agonists.

15 Administration of the prenyl-protein transferase inhibitor may also be combined with surgical treatment of endometriosis (such as surgical removal of misplaced endometrial tissue) where appropriate.

20 The instant compounds may also be useful as inhibitors of corneal inflammation. These compounds may improve the treatment of corneal opacity which results from cauterization-induced corneal inflammation. The instant compounds may also be useful in reducing corneal edema and neovascularization. (K. Sonoda et al., *Invest. Ophthalmol. Vis. Sci.*, 1998, vol. 39, p 2245-2251).

25 The compounds of this invention may be administered to mammals, preferably humans, either alone or, preferably, in combination with pharmaceutically acceptable carriers, excipients or diluents, in a pharmaceutical composition, according to standard pharmaceutical practice. The compounds can be administered orally or parenterally, including the intravenous, intramuscular, intraperitoneal, subcutaneous, rectal and topical routes of administration.

30 Additionally, the compounds of the instant invention may be administered to a mammal in need thereof using a gel extrusion mechanism (GEM) device, such as that described in U.S. Serial No. 60/144,643, filed on July 20, 1999, which is hereby incorporated by reference.

As used herein, the term "composition" is intended to encompass a product comprising the specified ingredients in the specific amounts, as well as

any product which results, directly or indirectly, from combination of the specific ingredients in the specified amounts.

The pharmaceutical compositions containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous
5 or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preserving agents in order
10 to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for
15 example, microcrystalline cellulose, sodium crosscarmellose, corn starch, or alginic acid; binding agents, for example starch, gelatin, polyvinyl-pyrrolidone or acacia, and lubricating agents, for example, magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to mask the unpleasant taste of the drug or delay disintegration and absorption in the gastrointestinal tract and
20 thereby provide a sustained action over a longer period. For example, a water soluble taste masking material such as hydroxypropyl-methylcellulose or hydroxypropyl-cellulose, or a time delay material such as ethyl cellulose, cellulose acetate butyrate may be employed.

Formulations for oral use may also be presented as hard gelatin
25 capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water soluble carrier such as polyethyleneglycol or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

30 Aqueous suspensions contain the active material in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethyl-cellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally-occurring
35 phosphatide, for example lecithin, or condensation products of an alkylene oxide

with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethylene-oxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or
5 condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose, saccharin or aspartame.

10 Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavoring agents may be added to provide
15 a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as butylated hydroxyanisol or alpha-tocopherol.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more
20 preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

The pharmaceutical compositions of the invention may also be
25 in the form of an oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring phosphatides, for example soy bean lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate, and
30 condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening, flavoring agents, preservatives and antioxidants.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also
35 contain a demulcent, a preservative, flavoring and coloring agents and antioxidant.

The pharmaceutical compositions may be in the form of a sterile injectable aqueous solutions. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution.

5 The sterile injectable preparation may also be a sterile injectable oil-in-water microemulsion where the active ingredient is dissolved in the oily phase. For example, the active ingredient may be first dissolved in a mixture of soybean oil and lecithin. The oil solution then introduced into a water and glycerol mixture and processed to form a microemulsion.

10 The injectable solutions or microemulsions may be introduced into a patient's blood-stream by local bolus injection. Alternatively, it may be advantageous to administer the solution or microemulsion in such a way as to maintain a constant circulating concentration of the instant compound. In order to maintain such a constant concentration, a continuous intravenous delivery device may be utilized. An example of such a device is the Deltec CADD-PLUS™ model 5400 intravenous
15 pump.

The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension for intramuscular and subcutaneous administration. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been
20 mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butane diol. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides.
25 In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Compounds of Formula I may also be administered in the form of a suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in
30 the rectum to release the drug. Such materials include cocoa butter, glycerinated gelatin, hydrogenated vegetable oils, mixtures of polyethylene glycols of various molecular weights and fatty acid esters of polyethylene glycol.

For topical use, creams, ointments, jellies, solutions or suspensions, etc., containing the compound of Formula I are employed. (For purposes of this
35 application, topical application shall include mouthwashes and gargles.)

The compounds for the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles and delivery devices, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in the art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen. Compounds of the present invention may also be delivered as a suppository employing bases such as cocoa butter, glycerinated gelatin, hydrogenated vegetable oils, mixtures of polyethylene glycols of various molecular weights and fatty acid esters of polyethylene glycol.

When a compound according to this invention is administered into a human subject, the daily dosage will normally be determined by the prescribing physician with the dosage generally varying according to the age, weight, sex and response of the individual patient, as well as the severity of the patient's symptoms.

In one exemplary application, a suitable amount of compound is administered to a mammal undergoing treatment for cancer. Administration occurs in an amount between about 0.1 mg/kg of body weight to about 60 mg/kg of body weight per day, preferably of between 0.5 mg/kg of body weight to about 40 mg/kg of body weight per day.

The compounds of the instant invention may also be co-administered with other well known therapeutic agents that are selected for their particular usefulness against the condition that is being treated. For example, the compounds of the instant invention may also be co-administered with other well known cancer therapeutic agents that are selected for their particular usefulness against the condition that is being treated. Included in such combinations of therapeutic agents are combinations of the instant prenyl-protein transferase inhibitors and an antineoplastic agent. It is also understood that such a combination of antineoplastic agent and inhibitor of prenyl-protein transferase may be used in conjunction with other methods of treating cancer and/or tumors, including radiation therapy and surgery. It is further understood that any of the therapeutic agents described herein may also be used in combination with a compound of the instant invention and an antineoplastic agent.

Examples of an antineoplastic agent include, in general, microtubule-stabilizing agents such as paclitaxel (also known as Taxol®), docetaxel (also known as Taxotere®), epothilone A, epothilone B, desoxyepothilone A, desoxyepothilone

B or their derivatives); microtubule-disruptor agents; alkylating agents, for example, nitrogen mustards, ethyleneimine compounds, alkyl sulfonates and other compounds with an alkylating action such as nitrosoureas, cisplatin, and dacarbazine; anti-metabolites, for example, folic acid, purine or pyrimidine antagonists; 5 epidophyllotoxin; an antineoplastic enzyme; a topoisomerase inhibitor; procarbazine; mitoxantrone; platinum coordination complexes; biological response modifiers and growth inhibitors; mitotic inhibitors, for example, vinca alkaloids and derivatives of podophyllotoxin; cytotoxic antibiotics; hormonal/anti-hormonal therapeutic agents, haematopoietic growth factors and antibodies (such as trastuzumab, also known as 10 Herceptin™).

Example classes of antineoplastic agents include, for example, the anthracycline family of drugs, the vinca drugs, the mitomycins, the bleomycins, the cytotoxic nucleosides, the taxanes, the epothilones, discodermolide, the pteridine family of drugs, diynenes and the podophyllotoxins. Particularly useful members 15 of those classes include, for example, doxorubicin, carminomycin, daunorubicin, aminopterin, methotrexate, methopterin, dichloro-methotrexate, mitomycin C, porfiromycin, 5-fluorouracil, 6-mercaptopurine, gemcitabine, cytosine arabinoside, podophyllotoxin or podo-phyllotoxin derivatives such as etoposide, etoposide phosphate or teniposide, melphalan, vinblastine, vincristine, leurosine, vindesine, 20 leurosine, paclitaxel and the like. Other useful antineoplastic agents include estramustine, cisplatin, carboplatin, cyclophosphamide, bleomycin, tamoxifen, ifosamide, melphalan, hexamethyl melamine, thiotepa, cytarabin, idatrexate, trimetrexate, dacarbazine, L-asparaginase, dactinomycin, mechlorethamine (nitrogen mustard), streptozocin, cyclophosphamide, carmustine (BCNU), lomustine 25 (CCNU), procarbazine, mitomycin, cytarabine, etoposide, methotrexate, bleomycin, chlorambucil, camptothecin, CPT-11, topotecan, ara-C, bicalutamide, flutamide, leuprolide, pyridobenzoindole derivatives, interferons and interleukins. Particular examples of antineoplastic, or chemotherapeutic, agents are described, for example, by D. J. Stewart in "Nausea and Vomiting: Recent Research and Clinical Advances", 30 Eds. J. Kucharczyk, et al., CRC Press Inc., Boca Raton, Florida, USA (1991), pages 177-203, especially page 188. See also, R. J. Gralla, et al., Cancer Treatment Reports, 68(1), 163-172 (1984).

The preferred class of antineoplastic agents is the taxanes and the preferred antineoplastic agent is paclitaxel.

The compounds of the instant invention may also be co-administered with antisense oligonucleotides which are specifically hybridizable with RNA or DNA deriving from human *ras* gene. Such antisense oligonucleotides are described in U.S. Patent No. 5,576,208 and PCT Publication No. WO 99/22772. The instant
5 compounds are particularly useful when co-administered with the antisense oligonucleotide comprising the amino acid sequence of SEQ.ID.NO: 2 of U.S. Patent No. 5,576,208.

Certain compounds of the instant invention may exhibit very low plasma concentrations and significant inter-individual variation in the plasma levels
10 of the compound. It is believed that very low plasma concentrations and high inter-subject variability achieved following administration of certain prenyl-protein transferase inhibitors to mammals may be due to extensive metabolism by cytochrome P450 enzymes prior to entry of drug into the systemic circulation. Prenyl-protein transferase inhibitors may be metabolized by cytochrome P450 enzyme
15 systems, such as CYP3A4, CYP2D6, CYP2C9, CYP2C19 or other cytochrome P450 isoform. If a compound of the instant invention demonstrates an affinity for one or more of the cytochrome P450 enzyme systems, another compound with a higher affinity for the P450 enzyme(s) involved in metabolism should be administered concomitantly. Examples of compounds that have a comparatively very high affinity
20 for CYP3A4, CYP2D6, CYP2C9, CYP2C19 or other P450 isoform include, but are not limited to, piperonyl butoxide, troleandomycin, erythromycin, proadifen, isoniazid, allylisopropylacetamide, ethinylestradiol, chloramphenicol, 2-ethynyl-naphthalene and the like. Such a high affinity compound, when employed in combination with a compound of formula I, may reduce the inter-individual variation
25 and increase the plasma concentration of a compound of formula I to a level having substantial therapeutic activity by inhibiting the metabolism of the compound of formula I. Additionally, inhibiting the metabolism of a compound of the instant invention prolongs the pharmacokinetic half-life, and thus the pharmacodynamic effect, of the compound.

30 A compound of the present invention may be employed in conjunction with antiemetic agents to treat nausea or emesis, including acute, delayed, late-phase, and anticipatory emesis, which may result from the use of a compound of the present invention, alone or with radiation therapy. For the prevention or treatment of emesis a compound of the present invention may be used in conjunction with other
35 anti-emetic agents, especially neurokinin-1 receptor antagonists, 5HT3 receptor

antagonists, such as ondansetron, granisetron, tropisetron, and zatisetron, GABAB receptor agonists, such as baclofen, or a corticosteroid such as Decadron (dexamethasone), Kenalog, Aristocort, Nasalide, Preferid, Benecorten or others such as disclosed in U.S. Patent Nos. 2,789,118, 2,990,401, 3,048,581, 3,126,375, 3,929,768,
5 3,996,359, 3,928,326 and 3,749,712. For the treatment or prevention of emesis, conjunctive therapy with a neurokinin-1 receptor antagonist, a 5HT₃ receptor antagonist and a corticosteroid is preferred.

Neurokinin-1 receptor antagonists of use in conjunction with the compounds of the present invention are fully described, for example, in U.S. Patent
10 Nos. 5,162,339, 5,232,929, 5,242,930, 5,373,003, 5,387,595, 5,459,270, 5,494,926, 5,496,833, 5,637,699, 5,719,147; European Patent Publication Nos. EP 0 360 390, 0 394 989, 0 428 434, 0 429 366, 0 430 771, 0 436 334, 0 443 132, 0 482 539, 0 498 069, 0 499 313, 0 512 901, 0 512 902, 0 514 273, 0 514 274, 0 514 275, 0 514 276, 0 515 681, 0 517 589, 0 520 555, 0 522 808, 0 528 495, 0 532 456,
15 0 533 280, 0 536 817, 0 545 478, 0 558 156, 0 577 394, 0 585 913, 0 590 152, 0 599 538, 0 610 793, 0 634 402, 0 686 629, 0 693 489, 0 694 535, 0 699 655, 0 699 674, 0 707 006, 0 708 101, 0 709 375, 0 709 376, 0 714 891, 0 723 959, 0 733 632 and 0 776 893; PCT International Patent Publication Nos. WO 90/05525, 90/05729, 91/09844, 91/18899, 92/01688, 92/06079, 92/12151, 92/15585, 92/17449,
20 92/20661, 92/20676, 92/21677, 92/22569, 93/00330, 93/00331, 93/01159, 93/01165, 93/01169, 93/01170, 93/06099, 93/09116, 93/10073, 93/14084, 93/14113, 93/18023, 93/19064, 93/21155, 93/21181, 93/23380, 93/24465, 94/00440, 94/01402, 94/02461, 94/02595, 94/03429, 94/03445, 94/04494, 94/04496, 94/05625, 94/07843, 94/08997, 94/10165, 94/10167, 94/10168, 94/10170, 94/11368, 94/13639, 94/13663, 94/14767,
25 94/15903, 94/19320, 94/19323, 94/20500, 94/26735, 94/26740, 94/29309, 95/02595, 95/04040, 95/04042, 95/06645, 95/07886, 95/07908, 95/08549, 95/11880, 95/14017, 95/15311, 95/16679, 95/17382, 95/18124, 95/18129, 95/19344, 95/20575, 95/21819, 95/22525, 95/23798, 95/26338, 95/28418, 95/30674, 95/30687, 95/33744, 96/05181, 96/05193, 96/05203, 96/06094, 96/07649, 96/10562, 96/16939, 96/18643, 96/20197,
30 96/21661, 96/29304, 96/29317, 96/29326, 96/29328, 96/31214, 96/32385, 96/37489, 97/01553, 97/01554, 97/03066, 97/08144, 97/14671, 97/17362, 97/18206, 97/19084, 97/19942 and 97/21702; and in British Patent Publication Nos. 2 266 529, 2 268 931, 2 269 170, 2 269 590, 2 271 774, 2 292 144, 2 293 168, 2 293 169, and 2 302 689.
The preparation of such compounds is fully described in the aforementioned patents
35 and publications.

A particularly preferred neurokinin-1 receptor antagonist for use in conjunction with the compounds of the present invention is 2-(R)-(1-(R)-(3,5-bis(trifluoromethyl)phenyl)ethoxy)-3-(S)-(4-fluorophenyl)-4-(3-(5-oxo-1H,4H-1,2,4-triazolo)methyl)morpholine, or a pharmaceutically acceptable salt thereof, which is
5 described in U.S. Patent No. 5,719,147.

For the treatment of cancer, it may be desirable to employ a compound of the present invention in conjunction with another pharmacologically active agent(s). A compound of the present invention and the other pharmacologically active agent(s) may be administered to a patient simultaneously, sequentially
10 or in combination. For example, the present compound may employed directly in combination with the other active agent(s), or it may be administered prior, concurrent or subsequent to the administration of the other active agent(s). In general, the currently available dosage forms of the known therapeutic agents for use in such combinations will be suitable.

For example, a compound of the present invention may be presented together with another therapeutic agent in a combined preparation, such as with an antiemetic agent for simultaneous, separate, or sequential use in the relief of emesis associated with employing a compound of the present invention and radiation therapy. Such combined preparations may be, for example, in the form of a twin pack. A
15 20 preferred combination comprises a compound of the present invention with antiemetic agents, as described above.

Radiation therapy, including x-rays or gamma rays which are delivered from either an externally applied beam or by implantation of tiny radioactive sources, may also be used in combination with the instant inhibitor of prenyl-protein
25 transferase alone to treat cancer.

Additionally, compounds of the instant invention may also be useful as radiation sensitizers, as described in WO 97/38697, published on October 23, 1997, and herein incorporated by reference.

The instant compounds may also be useful in combination with
30 other inhibitors of parts of the signaling pathway that links cell surface growth factor receptors to nuclear signals initiating cellular proliferation. Thus, the instant compounds may be utilized in combination with farnesyl pyrophosphate competitive inhibitors of the activity of farnesyl-protein transferase or in combination with a compound which has Raf antagonist activity. The instant compounds may also be

co-administered with compounds that are selective inhibitors of geranylgeranyl protein transferase.

In particular, if the compound of the instant invention is a selective inhibitor of farnesyl-protein transferase, co-administration with a compound(s) that is a selective inhibitor of geranylgeranyl protein transferase may provide an improved therapeutic effect.

In particular, the compounds disclosed in the following patents and publications may be useful as farnesyl pyrophosphate-competitive inhibitor component of the instant composition: U.S. Serial Nos. 08/254,228 and 08/435,047. Those patents and publications are incorporated herein by reference.

In practicing methods of this invention, which comprise administering, simultaneously or sequentially or in any order, two or more of a protein substrate-competitive inhibitor and a farnesyl pyrophosphate-competitive inhibitor, such administration can be orally or parenterally, including intravenous, intramuscular, intraperitoneal, subcutaneous, rectal and topical routes of administration. It is preferred that such administration be orally. It is more preferred that such administration be orally and simultaneously. When the protein substrate-competitive inhibitor and farnesyl pyrophosphate-competitive inhibitor are administered sequentially, the administration of each can be by the same method or by different methods.

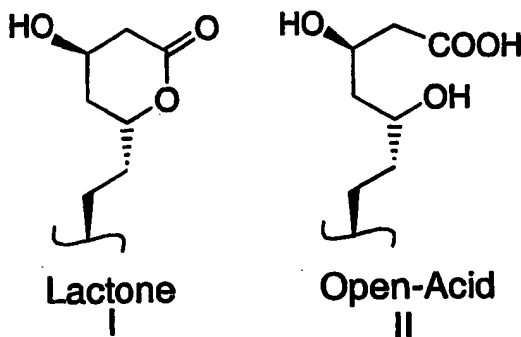
The instant compounds may also be useful in combination with an integrin antagonist for the treatment of cancer, as described in U.S. Serial No. 09/055,487, filed April 6, 1998, and WO 98/44797, published on October 15, 1998, which are incorporated herein by reference.

As used herein the term an integrin antagonist refers to compounds which selectively antagonize, inhibit or counteract binding of a physiological ligand to an integrin(s) that is involved in the regulation of angiogenesis, or in the growth and invasiveness of tumor cells. In particular, the term refers to compounds which selectively antagonize, inhibit or counteract binding of a physiological ligand to the $\alpha v \beta 3$ integrin, which selectively antagonize, inhibit or counteract binding of a physiological ligand to the $\alpha v \beta 5$ integrin, which antagonize, inhibit or counteract binding of a physiological ligand to both the $\alpha v \beta 3$ integrin and the $\alpha v \beta 5$ integrin, or which antagonize, inhibit or counteract the activity of the particular integrin(s) expressed on capillary endothelial cells. The term also refers to antagonists of the $\alpha 1 \beta 1$, $\alpha 2 \beta 1$, $\alpha 5 \beta 1$, $\alpha 6 \beta 1$ and $\alpha 6 \beta 4$ integrins. The term also refers to antagonists

of any combination of $\alpha v\beta 3$ integrin, $\alpha v\beta 5$ integrin, $\alpha 1\beta 1$, $\alpha 2\beta 1$, $\alpha 5\beta 1$, $\alpha 6\beta 1$ and $\alpha 6\beta 4$ integrins. The instant compounds may also be useful with other agents that inhibit angiogenesis and thereby inhibit the growth and invasiveness of tumor cells, including, but not limited to angiostatin and endostatin.

5 The instant compounds may also be useful in combination with an inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) for the treatment of cancer. Compounds which have inhibitory activity for HMG-CoA reductase can be readily identified by using assays well-known in the art. For example, see the assays described or cited in U.S. Patent No. 4,231,938 at col. 6,
10 and WO 84/02131 at pages 30-33. The terms "HMG-CoA reductase inhibitor" and "inhibitor of HMG-CoA reductase" have the same meaning when used herein.

 Examples of HMG-CoA reductase inhibitors that may be used include but are not limited to lovastatin (MEVACOR®; see US Patent No. 4,231,938; 4,294,926 and 4,319,039), simvastatin (ZOCOR®; see US Patent No. 4,444,784;
15 4,820,850 and 4,916,239), pravastatin (PRAVACHOL®; see US Patent Nos. 4,346,227; 4,537,859; 4,410,629; 5,030,447 and 5,180,589), fluvastatin (LESCOL®; see US Patent Nos. 5,354,772; 4,911,165; 4,929,437; 5,189,164; 5,118,853; 5,290,946 and 5,356,896), atorvastatin (LIPITOR®; see US Patent Nos. 5,273,995; 4,681,893; 5,489,691 and 5,342,952) and cerivastatin (also known as rivastatin and
20 BAYCHOL®; see US Patent No. 5,177,080). The structural formulas of these and additional HMG-CoA reductase inhibitors that may be used in the instant methods are described at page 87 of M. Yalpani, "Cholesterol Lowering Drugs", *Chemistry & Industry*, pp. 85-89 (5 February 1996) and US Patent Nos. 4,782,084 and 4,885,314. The term HMG-CoA reductase inhibitor as used herein includes all pharmaceutically
25 acceptable lactone and open-acid forms (i.e., where the lactone ring is opened to form the free acid) as well as salt and ester forms of compounds which have HMG-CoA reductase inhibitory activity, and therefor the use of such salts, esters, open-acid and lactone forms is included within the scope of this invention. An illustration of the lactone portion and its corresponding open-acid form is shown below as structures I
30 and II.



In HMG-CoA reductase inhibitors where an open-acid form can exist, salt and ester forms may preferably be formed from the open-acid, and all such forms are included within the meaning of the term "HMG-CoA reductase inhibitor" as used herein. Preferably, the HMG-CoA reductase inhibitor is selected from lovastatin and simvastatin, and most preferably simvastatin. Herein, the term "pharmaceutically acceptable salts" with respect to the HMG-CoA reductase inhibitor shall mean non-toxic salts of the compounds employed in this invention which are generally prepared by reacting the free acid with a suitable organic or inorganic base, particularly those formed from cations such as sodium, potassium, aluminum, calcium, lithium, magnesium, zinc and tetramethylammonium, as well as those salts formed from amines such as ammonia, ethylenediamine, N-methylglucamine, lysine, arginine, ornithine, choline, N,N'-dibenzylethylenediamine, chloroprocaine, diethanolamine, procaine, N-benzylphenethylamine, 1-p-chlorobenzyl-2-pyrrolidine-1'-yl-methylbenzimidazole, diethylamine, piperazine, and tris(hydroxymethyl) aminomethane. Further examples of salt forms of HMG-CoA reductase inhibitors may include, but are not limited to, acetate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, bromide, calcium edetate, camsylate, carbonate, chloride, clavulanate, citrate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycolylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynapthoate, iodide, isothionate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, methylsulfate, mucate, napsylate, nitrate, oleate, oxalate, pamaote, palmitate, panthothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, triethiodide, and valerate.

Ester derivatives of the described HMG-CoA reductase inhibitor compounds may act as prodrugs which, when absorbed into the bloodstream of a

warm-blooded animal, may cleave in such a manner as to release the drug form and permit the drug to afford improved therapeutic efficacy.

Similarly, the instant compounds may be useful in combination with agents that are effective in the treatment and prevention of NF-1, restenosis, polycystic kidney disease, infections of hepatitis delta and related viruses and fungal infections.

If formulated as a fixed dose, such combination products employ the combinations of this invention within the dosage range described above and the other pharmaceutically active agent(s) within its approved dosage range.

Combinations of the instant invention may alternatively be used sequentially with known pharmaceutically acceptable agent(s) when a multiple combination formulation is inappropriate.

The instant compounds may also be useful in combination with prodrugs of antineoplastic agents. In particular, the instant compounds may be co-administered either concurrently or sequentially with a conjugate (termed a "PSA conjugate") which comprises an oligopeptide, that is selectively cleaved by enzymatically active prostate specific antigen (PSA), and an antineoplastic agent. Such co-administration will be particularly useful in the treatment of prostate cancer or other cancers which are characterized by the presence of enzymatically active PSA in the immediate surrounding cancer cells, which is secreted by the cancer cells.

Compounds which are PSA conjugates and are therefore useful in such a co-administration, and methods of synthesis thereof, can be found in the following patents, pending patent applications and publications which are herein incorporated by references:

U.S. Patent No. 5,599,686, granted on February 4, 1997;
WO 96/00503 (January 11, 1996); US Serial No. 08/404,833, filed on March 15, 1995; US Serial No. 08/468,161, filed on June 6, 1995;
U.S. Patent No. 5,866,679, granted on February 2, 1999;
U.S. Patent No. 5,998,362, granted on December 7, 1999;
U.S. Patent No. 5,948,750, granted on September 7, 1999;
WO 99/02175 (January 21, 1999); US Serial No. 09/112,656, filed on July 9, 1998;
and
WO 99/28345 (June 10, 1999); US Serial No. 09/193,365, filed on November 17, 1998.

Compounds which are described as prodrugs wherein the active therapeutic agent is released by the action of enzymatically active PSA and therefore may be useful in such a co-administration, and methods of synthesis thereof, can be found in the following patents, pending patent applications and publications, which
5 are herein incorporated by reference: WO 98/52966 (November 26, 1998).

All patents, publications and pending patent applications identified are herein incorporated by reference.

The compounds of the instant invention are also useful as a component in an assay to rapidly determine the presence and quantity of farnesyl-protein transferase (FPTase) in a composition. Thus the composition to be tested may be divided
10 and the two portions contacted with mixtures which comprise a known substrate of FPTase (for example a tetrapeptide having a cysteine at the amine terminus) and farnesyl pyrophosphate and, in one of the mixtures, a compound of the instant invention. After the assay mixtures are incubated for a sufficient period of time,
15 well known in the art, to allow the FPTase to farnesylate the substrate, the chemical content of the assay mixtures may be determined by well known immuno-logical, radiochemical or chromatographic techniques. Because the compounds of the instant invention are selective inhibitors of FPTase, absence or quantitative reduction of the amount of substrate in the assay mixture without the compound of the instant
20 invention relative to the presence of the unchanged substrate in the assay containing the instant compound is indicative of the presence of FPTase in the composition to be tested.

It would be readily apparent to one of ordinary skill in the art that such an assay as described above would be useful in identifying tissue samples
25 which contain farnesyl-protein transferase and quantitating the enzyme. Thus, potent inhibitor compounds of the instant invention may be used in an active site titration assay to determine the quantity of enzyme in the sample. A series of samples composed of aliquots of a tissue extract containing an unknown amount of farnesyl-protein transferase, an excess amount of a known substrate of FPTase (for example a
30 tetrapeptide having a cysteine at the amine terminus) and farnesyl pyrophosphate are incubated for an appropriate period of time in the presence of varying concentrations of a compound of the instant invention. The concentration of a sufficiently potent inhibitor (i.e., one that has a K_i substantially smaller than the concentration of enzyme in the assay vessel) required to inhibit the enzymatic activity of the sample by 50%

is approximately equal to half of the concentration of the enzyme in that particular sample.

EXAMPLES

5

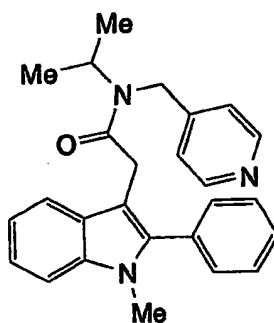
Examples provided are intended to assist in a further understanding of the invention. Particular materials employed, species and conditions are intended to be further illustrative of the invention and are not intended to limit the reasonable scope thereof.

10

EXAMPLE 1

Preparation of N-isopropyl-2-(1-methyl-2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide

15



Step A: Preparation of N-(pyridin-4-ylmethyl)propan-2-amine

To a solution of pyridine-4-carboxaldehyde (0.53 g, 4.95mmol) in 20 5 mL of MeOH was added isopropylamine (0.421 mL, 4.95 mmol). The resulting solution was stirred at room temperature for 25 minutes, then cooled to 0°C. Solid sodium borohydride (0.206 g, 5.44 mmol) was added, and the reaction was stirred at room temperature for 80 minutes. Saturated aqueous ammonium chloride was added to the reaction, and the resulting mixture was stirred until gas evolution ceased. 25 The mixture was partitioned between CH₂Cl₂ and saturated aqueous sodium bicarbonate, and the aqueous solution was extracted with CH₂Cl₂ (7 x). The combined organic

solutions were dried (Na_2SO_4), filtered, and concentrated *in vacuo* to provide a clear oil. ES mass spectrum m/e 151 ($M+1$).

Step B: Preparation of N-isopropyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide

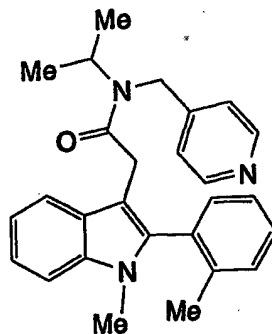
5
A solution of N-(pyridin-4-ylmethyl)propan-2-amine (0.650 g) and 2-phenylindole-3-acetic acid (1.09 g, 4.33 mmol) in 10 mL of THF and 5 mL of DMF was cooled to 0°C . EDC (0.913 g, 4.76 mmol) was added in one portion. The reaction was stirred at room temperature for 6 h, then partitioned between EtOAc and 1 N aqueous NaHSO_4 . The aqueous solution was basified by addition of sodium carbonate, then extracted with EtOAc (4 x). The combined organic solutions were dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Flash chromatography (linear gradient, 10-40% MeOH/EtOAc) afforded the title compound as a white foam. Exact mass calcd for $\text{C}_{25}\text{H}_{25}\text{N}_3\text{O}$: 384.2071; found: 384.2076 (FAB).

15
Step C: Preparation of N-isopropyl-2-(1-methyl-2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide

The indole described in Step B above (0.500 g, 0.261 mmol) was added to a suspension of 18-crown-6 (0.007 g, 0.03 mmol) and KOtBu (0.044 g, 0.39 mmol) in 3 mL of Et_2O . The resulting orange suspension was stirred at room temperature for 15 minutes. Methyl iodide (0.024 mL, 0.39 mmol) was added via syringe, and the reaction was stirred at room temperature for 3 hours 20 minutes. The mixture was then partitioned between EtOAc and water. The aqueous solution was extracted with EtOAc (2 x). The combined organic solutions were dried (25
 Na_2SO_4), filtered, and concentrated *in vacuo*. Flash chromatography (linear gradient, 99% CH_2Cl_2 /0.9% MeOH/0.1% aqueous NH_4OH - 97% CH_2Cl_2 /3.6% MeOH/0.4% aqueous NH_4OH) afforded the title compound, which was isolated as its hydrochloride salt. ES mass spectrum m/e 398 ($M+1$).

30
EXAMPLE 2

Preparation of N-Isopropyl-2-(1-methyl-2-*o*-tolyl-1H-indol-3-yl)-N-pyridin-4-ylmethyl-acetamide



Step A: Preparation of (2-Bromo-1H-indol-3-yl)-acetonitrile

To a solution of indole-3-acetonitrile (13.0 g, 83.23 mmol) and silica gel (13 g) in 130 ml of dry CH₂Cl₂ at 0°C was added NBS (14.8 g, 83.23 mmol). The ice bath was then removed and the reaction stirred at room temperature for 16 hours. The solution was filtered, and the liquid filtrate was washed with 5% aqueous sodium metabisulfite (3 x), then dried (MgSO₄), filtered, and concentrated *in vacuo*. Flash chromatography (5%-30% EtOAc/hexane) provided the titled product.

Step B: Preparation of (2-*o*-Tolyl-1H-indol-3-yl)-acetonitrile

To a solution of (2-bromo-1H-indol-3-yl)-acetonitrile (200 mg, .851 mmol) and *ortho*-tolyl boronic acid (174 mg, 1.28 mmol) in 12 ml of toluene and 12 ml of ethanol was added 1 M aqueous Na₂CO₃ (2.13 ml, 2.13 mmol), Pd(PPh₃)₄ (49.2 mg, .04 mmol), and LiCl (108.2 mg, 2.55 mmol). The reaction was heated to reflux for 3 hours then cooled to room temperature and stirred for 14 hours. Upon completion the reaction was concentrated *in vacuo*. Purification by silica gel chromatography (0-20% EtOAc/hexane) provided the titled product.

Step C: Preparation of (2-*o*-Tolyl-1H-indol-3-yl)-acetic acid

To a solution of 2-*ortho*-tolyl-1H-indol-3-yl)-acetonitrile (0.196 g, 0.796 mmol) in 5 ml of MeOH was added 5 ml of 40% aqueous NaOH. The reaction was heated to reflux for 16 hours, then cooled to room temperature. The solution was then acidified with 3 N aqueous HCl and washed with CH₂Cl₂ (3 x). The solution was dried (MgSO₄), filtered, and concentrated *in vacuo* to provide the titled product.

Step D: Preparation of N-Isopropyl-N-pyridin-4-ylmethyl-2-(2-*o*-tolyl-1H-indol-3-yl)-acetamide

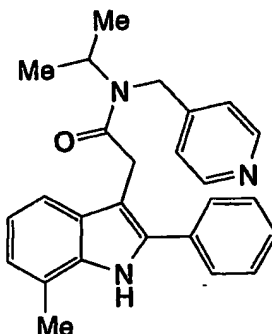
To a solution of (2-*ortho*-tolyl-1H-indol-3-yl)-acetic acid (0.100 g, 0.377 mmol) in 0.800 ml of DMF was N-(pyridin-4-ylmethyl)propan-2-amine (0.056 g, 0.38 mmol), DIEA (0.200 mL, 1.13 mmol), and PyBOP (0.216 g, 0.410 mmol). The solution stirred at room temperature for 3 days. Upon completion of the reaction, the solution was purified by reverse phase chromatography to provide the titled product.

Step E: Preparation of N-Isopropyl-2-(1-methyl-2-*o*-tolyl-1H-indol-3-yl)-N-pyridin-4-ylmethyl-acetamide

To a suspension of 18-crown-6 (0.002 g, 0.01 mmol) and KOtBu (0.010 g, 0.09 mmol) in Et₂O (1 mL) was added N-Isopropyl-N-pyridin-4-ylmethyl-2-(2-*o*-tolyl-1H-indol-3-yl)-acetamide in one portion. After stirring at room temperature for 15 minutes, iodomethane (0.013 g, 0.090 mmol) was added. The reaction was stirred at room temperature for 24 hours and then quenched with water. The mixture was poured into EtOAc, washed with water, dried (MgSO₄), filtered and concentrated *in vacuo*. Purification by reverse phase HPLC yielded the titled product. ES mass spectrum *m/e* 412 (M+1).

EXAMPLE 3

Preparation of N-isopropyl-2-(7-methyl-2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide



Step A: Preparation of N-isopropyl-4-oxo-4-phenyl-N-(pyridin-4-ylmethyl)butanamide

To a solution of 4-oxo-4-phenylbutanoic acid (0.500 g, 2.81 mmol) in 3.5 mL of DMF at room temperature were added N-(pyridin-4-ylmethyl)propan-2-amine (0.464 g, 3.09 mmol) and EDC (0.591 g, 3.09 mmol). A white solid precipitated from the solution. The reaction mixture was stirred for 7 hours, then partitioned between EtOAc and saturated aqueous sodium bicarbonate. The aqueous solution was extracted with EtOAc (2 x). The combined organic solutions were washed with 1:1 saturated aqueous sodium chloride:water (1 x) and saturated aqueous sodium chloride (1 x), then dried over sodium sulfate, filtered, and concentrated. Flash chromatography (linear gradient, 99% CH₂Cl₂/0.9% MeOH/0.1% aqueous NH₄OH - 97% CH₂Cl₂/2.7% MeOH/0.3% aqueous NH₄OH) afforded the title compound.

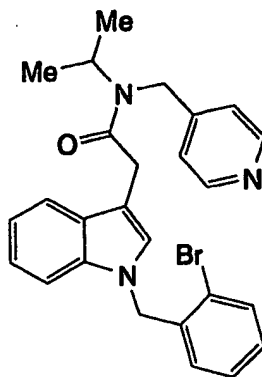
Step B: Preparation of N-isopropyl-2-(7-methyl-2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide

The ketone prepared in Step A (0.051 g, 0.164 mmol) was dissolved in 1 mL of toluene. *Ortho*-Tolylhydrazine hydrochloride (0.026 g, 0.164 mmol) was added in one portion, and the resulting suspension was heated to 100°C. After 1 hour, the reaction was cooled to room temperature and concentrated *in vacuo*. The unpurified hydrazone was combined with zinc chloride (0.112 g, 0.82 mmol), and the mixture was heated to 170°C. After 5 minutes, the reaction was cooled to room temperature and diluted with acetone to give a brown solution. The solution was partitioned between EtOAc and water. The organic solution was washed with saturated aqueous sodium chloride (1 x). The aqueous solutions were extracted with EtOAc (2 x), and the combined organic solutions were washed with saturated aqueous sodium chloride (1 x), then dried over sodium sulfate, filtered, and concentrated *in vacuo*. Purification by reversed phase HPLC provided the title compound as its trifluoroacetate salt. ES mass spectrum *m/e* 398 (M+1).

EXAMPLE 4

Preparation of 2-[1-(2-bromobenzyl)-1H-indol-3-yl]-N-isopropyl-N-(pyridin-4-ylmethyl)acetamide

5



Step A: Preparation of 2-(1H-indol-3-yl)-N-isopropyl-N-(pyridin-4-ylmethyl)acetamide

10

A solution of N-(pyridin-4-ylmethyl)propan-2-amine (4.93 g, 32.87 mmol) and indole-3-acetic acid (5.75 g, 32.81 mmol) in 48 mL of THF and 48 mL of DMF was cooled to 0°C. EDC (6.92 g, 36.10 mmol) was added in one portion. The reaction was stirred at room temperature for 15 hours, then partitioned between EtOAc and saturated aqueous sodium bicarbonate. The aqueous solution was extracted with EtOAc (2 x). The combined organic solutions were washed with saturated aqueous sodium chloride, dried (Na₂SO₄), filtered, and concentrated *in vacuo*. Flash chromatography (linear gradient, 98% CH₂Cl₂/1.8% MeOH/0.2% aqueous NH₄OH - 95% CH₂Cl₂/4.5% MeOH/0.5% aqueous NH₄OH) afforded a pink foam. ES mass spectrum m/e 308 (M+1).

20

Step B: Preparation of 2-[1-(2-bromobenzyl)-1H-indol-3-yl]-N-isopropyl-N-(pyridin-4-ylmethyl)acetamide

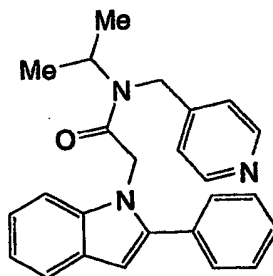
A 25 mL round-bottomed flask was charged with a 60% dispersion of sodium hydride in mineral oil (0.033 g, 0.81 mmol). The suspension was washed with hexanes (2 mL), and the flask was charged with 2.5 mL of DMF and 0.250g of the indole prepared in Step A. The suspension was stirred at room temperature

25

for 30 minutes to give a green solution. The solution was cooled to 0°C, and 2-bromobenzyl bromide (0.203 g, 0.81 mmol) was added in one portion. After 1 hour, the reaction was quenched with saturated aqueous ammonium chloride, then partitioned between EtOAc and saturated aqueous sodium bicarbonate. The aqueous solution was extracted with EtOAc (2 x). The combined organic solutions were washed with saturated aqueous sodium chloride, dried (Na₂SO₄), filtered, and concentrated *in vacuo*. Flash chromatography (linear gradient, 99% CH₂Cl₂/0.9% MeOH/0.1% aqueous NH₄OH - 97% CH₂Cl₂/2.7% MeOH/0.3% aqueous NH₄OH) afforded a white foam. Exact mass calcd for C₂₆H₂₆BrN₃O: 416.1332; found: 476.1323 (FAB).

EXAMPLE 5

Preparation of N-isopropyl-2-(2-phenyl-1H-indol-1-yl)-N-(pyridin-4-ylmethyl)acetamide



Step A: Preparation of (2-phenyl-1H-indol-1-yl)acetic acid

Freshly crushed KOH (2.69 g, 48.02 mmol) and DMSO were combined and stirred at room temperature for 5 minutes. 2-phenylindole (2.00 g, 10.35 mmol) was added in one portion, and the resulting mixture was stirred for 45 minutes. The reaction was cooled to 0°C, and methyl bromoacetate (1.96 mL, 20.70 mmol) was added via syringe. The reaction was stirred at room temperature for 8 hours 30 minutes, then poured into water. The mixture was extracted with EtOAc (2 x) to remove residual starting material. The pH of the aqueous solution was then adjusted to 4 by addition of conc. aqueous HCl, and the solution was extracted with EtOAc (2 x). The organic extracts were dried (Na₂SO₄), filtered, and concentrated

in vacuo to provide the title compound.

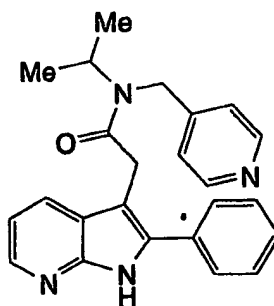
Step B: Preparation of N-isopropyl-2-(2-phenyl-1H-indol-1-yl)-N-(pyridin-4-ylmethyl)acetamide

5 To a solution of the acid from Step A above (0.050 g, 0.199 mmol) and N-(pyridin-4-ylmethyl)propan-2-amine (0.033 g, 0.220 mmol) in 1 mL of DMF were added diisopropylethylamine (0.069 mL, 0.400 mmol) and PYBOP (0.114g, 0.220 mmol). The reaction was stirred at room temperature for 15 hours, then purified directly by reverse phase HPLC (C₁₈, acetonitrile/water/TFA). Further purification
10 by flash chromatography (linear gradient, 99% CH₂Cl₂/0.9% MeOH/0.1% aqueous NH₄OH - 97% CH₂Cl₂/2.7% MeOH/0.3% aqueous NH₄OH) afforded the title compound as a white foam. Exact mass calcd for C₂₅H₂₅N₃O: 384.2070; found: 384.2054 (FAB).

15

EXAMPLE 6

Preparation of N-Isopropyl-2-(2-phenyl-1H-pyrrolo[2,3-b]pyridin-3-yl)-N-pyridin-4-ylmethyl-acetamide



20

Step A: Preparation of 2-Phenyl-3-pyrrolidin-1-ylmethyl-1H-pyrrolo[2,3-b]pyridine

25 A solution of 2-phenyl-1H-pyrrolo[2,3-b]pyridine (1.0 g, 5.15 mmol, prepared according to: Hands, D.; Bishop, B.; Cameron, M.; Edwards, J. S.; Cottrell, I. F.; Wright, S. H. B. *Synthesis* 1996, 877-882.), pyrrolidine (2.2 g, 30.89 mmol), and formaldehyde (37% by wt., 2.51g, 30.89 mmol) in HOAc/dioxane (2ml/8ml) was stirred at room temperature for 72 hours. The solution was acidified with 1ml of 3N

HCl/10ml H₂O and washed with ether. The water layer was then basified with solid K₂CO₃, and a white precipitate formed. The precipitate was washed with water and then with CH₂Cl₂ to yield the titled product.

5 Step B: Preparation of 2-Phenyl-1H-pyrrolo[2,3-b]pyridin-3-yl)-acetonitrile

To a solution of 2-phenyl-3-pyrrolidin-1-ylmethyl-1H-pyrrolo[2,3-b]pyridine in MeOH (7 ml) was added iodomethane (0.958 g, 6.75 mmol). The reaction stirred at room temperature for 3 hours. The MeOH was then removed *in vacuo*. The residue was dissolved in THF (10 ml) and treated with KCN (0.916 g, 14.06 mmol) in water. The reaction stirred at reflux for 16 hours. Additional KCN (0.549 g, 8.44 mmol) was added, and the reaction continued to stir at reflux for 72 hours. The reaction was cooled to room temperature, filtered, and washed with water to yield the titled product.

15 Step C: Preparation of (2-Phenyl-1H-pyrrolo[2,3-b]pyridin-3-yl)-acetic acid

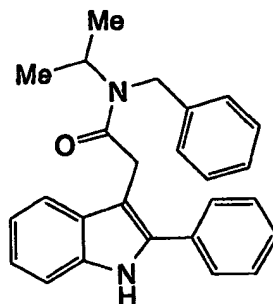
To a solution of 2-phenyl-1H-pyrrolo[2,3-b]pyridin-3-yl)-acetonitrile (0.703 g, 3.014 mmol) in 15 ml of MeOH was added 15 ml of 40% aqueous NaOH. The reaction was heated to reflux for 26 hours, then cooled to room temperature. The solution was acidified with 3 N HCl and washed with CH₂Cl₂ (3 x). The aqueous solution was then washed with *n*-butanol (3 x). Both CH₂Cl₂ and *n*-butanol solutions were combined and concentrated *in vacuo* to yield the titled product.

Step D: Preparation of N-Isopropyl-2-(2-phenyl-1H-pyrrolo[2,3-b]pyridin-3-yl)-N-pyridin-4-ylmethyl-acetamide

25 To a solution of (2-Phenyl-1H-pyrrolo[2,3-b]pyridin-3-yl)-acetic acid (0.200g, 0.793 mmol) in 1ml of DMSO was added N-(pyridin-4-ylmethyl)propan-2-amine (0.119 g, 0.79 mmol), DIEA (0.41 ml, 2.38 mmol), and PYBOP (0.454 mg, 0.87 mmol). The solution stirred at room temperature for 3 days. Upon completion the reaction was directly purified by reverse phase HPLC to provide the titled product. ES mass spectrum *m/e* 412 (M+1).

30

EXAMPLE 7

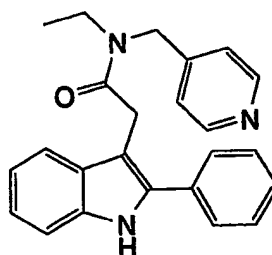
Preparation of N-benzyl-N-isopropyl-2-(2-phenyl-1H-indol-3-yl)acetamide

5

The above-titled compound was prepared according to the procedure of Example 1, Step B, using benzylisopropylamine in place of N-(pyridin-4-ylmethyl)propan-2-amine.

10

EXAMPLE 8

Preparation of N-ethyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide

15

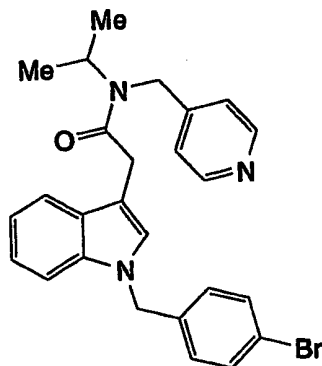
The above-titled compound was prepared according to the procedure of Example 1, Step B, using 4-(ethylaminomethyl)pyridine in place of N-(pyridin-4-ylmethyl)propan-2-amine.

20

EXAMPLE 9

Preparation of 2-[1-(4-bromobenzyl)-1H-indol-3-yl]-N-isopropyl-N-(pyridin-4-ylmethyl)acetamide

5



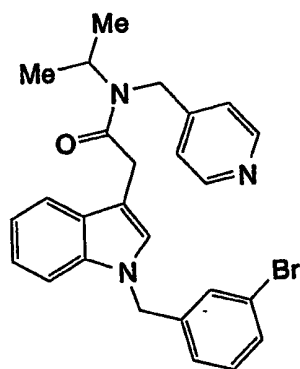
The above-titled compound was prepared according to the procedures of Example 4, Step B, using 4-bromobenzyl bromide in place of 2-bromobenzyl bromide. Exact mass calcd for $C_{26}H_{26}BrN_3O$: 476.1332; found: 476.1312 (FAB).

10

EXAMPLE 10

Preparation of 2-[1-(3-bromobenzyl)-1H-indol-3-yl]-N-isopropyl-N-(pyridin-4-ylmethyl)acetamide

15

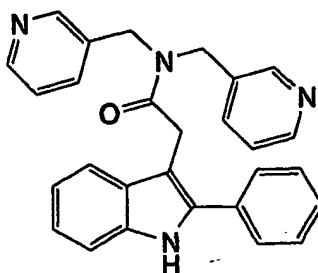


The above-titled compound was prepared according to the procedures of Example 4, Step B, using 3-bromobenzyl bromide in place of 2-bromobenzyl bromide. Exact mass calcd for $C_{26}H_{26}BrN_3O$: 476.1332; found: 476.1312 (FAB).

5

EXAMPLE 11

Preparation of 2-(2-phenyl-1H-indol-3-yl)-N,N-bis(pyridin-3-ylmethyl)acetamide



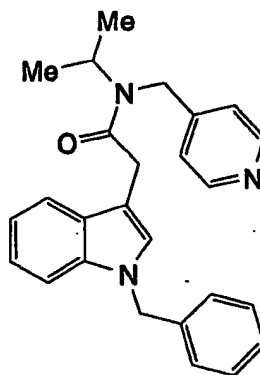
10

The above-titled compound was prepared according to the procedure of Example 1, Step B, using 3,3'-dipicolylamine in place of N-(pyridin-4-ylmethyl)propan-2-amine.

15

EXAMPLE 12

Preparation of 2-(1-benzyl-1H-indol-3-yl)-N-isopropyl-N-(pyridin-4-ylmethyl)acetamide



20

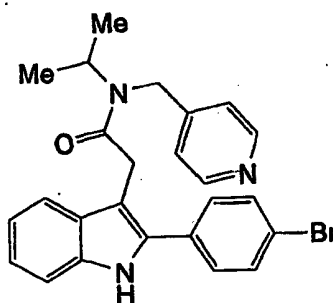
The above-titled compound was prepared according to the procedures of Example 4, Step B, using benzyl bromide in place of 2-bromobenzyl bromide. Exact mass calcd for $C_{26}H_{27}N_3O$: 398.2227; found: 398.2201 (FAB).

5

EXAMPLE 13

Preparation of 2-[2-(4-bromophenyl)-1H-indol-3-yl]-N-isopropyl-N-(pyridin-4-ylmethyl)acetamide

10



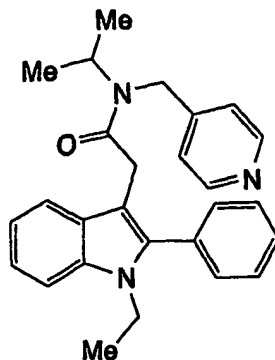
The above-titled compound was prepared according to the procedures of Example 3 using 3-(4-bromobenzoyl) propionic acid in place of 4-oxo-4-phenylbutanoic acid in Step A and phenylhydrazine hydrochloride in place of *ortho*-tolylhydrazine hydrochloride in Step B. ES mass spectrum m/e 462 ($M+1$).

15

EXAMPLE 14

20

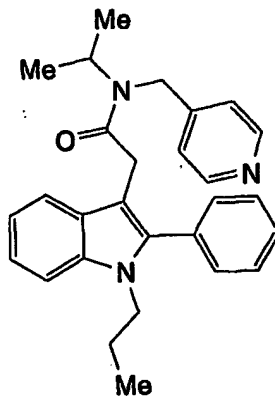
Preparation of 2-(1-ethyl-2-phenyl-1H-indol-3-yl)-N-isopropyl-N-(pyridin-4-ylmethyl)acetamide



The above-titled compound was prepared according to the procedure of Example 1, Step C using ethyl iodide in place of methyl iodide. Exact mass calcd for C₂₇H₂₉N₃O: 412.2383; found: 412.2368 (FAB).

EXAMPLE 15

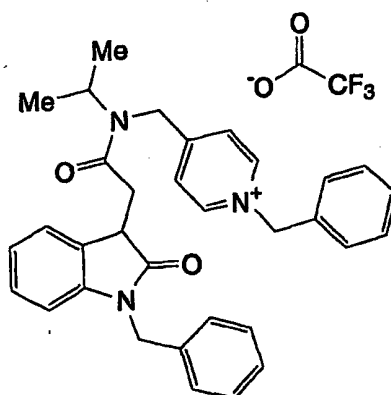
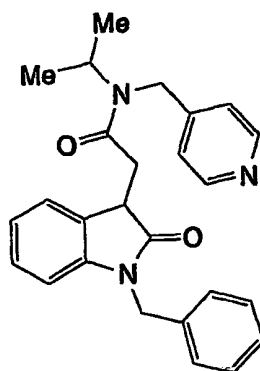
Preparation of N-isopropyl-2-(2-phenyl-1-propyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide



The above-titled compound was prepared according to the procedure of Example 1, Step C using *n*-propyl iodide in place of methyl iodide. Exact mass calcd for C₂₈H₃₁N₃O: 426.2540; found: 426.2537 (FAB).

EXAMPLE 16

Preparation of 2-(1-benzyl-2-oxo-2,3-dihydro-1H-indol-3-yl)-N-isopropyl-N-(pyridin-4-ylmethyl) acetamide and 1-benzyl-4-[[[(1-benzyl-2-oxo-2,3-dihydro-1H-indol-3-yl)acetyl] (isopropyl)amino]methyl]pyridinium trifluoroacetate



To a solution of 2-(1H-indol-3-yl)-N-isopropyl-N-(pyridin-4-ylmethyl) acetamide (0.050 g, 0.163 mmol) in 2 mL of DMSO was added solid KOH. The reaction was stirred for 15 minutes at room temperature, then benzyl bromide (0.031 g, 0.180 mmol) was added via syringe. After 5 hours stirring at room temperature, the reaction was acidified with conc. HCl and purified directly by reverse phase HPLC to give:

- (a) 2-(1-benzyl-2-oxo-2,3-dihydro-1H-indol-3-yl)-N-isopropyl-N-(pyridin-4-ylmethyl)-acetamide as its trifluoroacetate salt.

ES mass spectrum m/e 414 (M+1); and

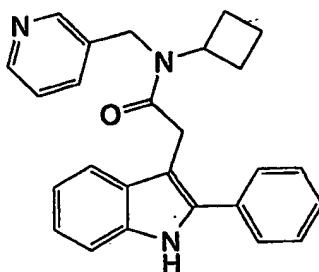
- 5 (b) 1-benzyl-4-[[[(1-benzyl-2-oxo-2,3-dihydro-1H-indol-3-yl)acetyl] (isopropyl)-amino]methyl]pyridinium trifluoroacetate.

ES mass spectrum m/e 414 (M+1).

EXAMPLE 17

10

Preparation of N-Cyclobutyl-2-(2-phenyl-1H-indol-3-yl)-N-pyridin-3-ylmethyl-acetamide



15

The above-titled compound was prepared according to the procedures of Example 1, Steps A and B, using pyridine-3-carboxaldehyde in place of pyridine-4-carboxaldehyde and cyclobutylamine in place of isopropylamine.

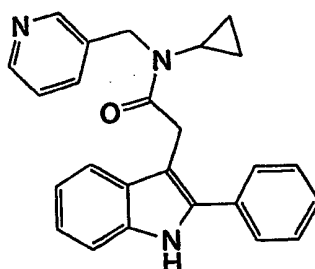
ES mass spectrum m/e 396 (M+1).

20

EXAMPLE 18

Preparation of N-Cyclopropyl-2-(2-phenyl-1H-indol-3-yl)-N-pyridin-3-ylmethyl-acetamide

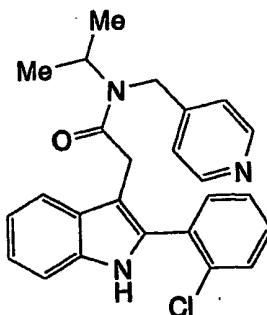
25



- The above-titled compound was prepared according to the procedures of Example 1, Steps A and B, using pyridine-3-carboxaldehyde in place of pyridine-4-carboxaldehyde and cyclopropylamine in place of isopropylamine. Exact mass calcd for $C_{25}H_{23}N_3O$: 381.1914; found: 382.1909 (FAB).

EXAMPLE 19

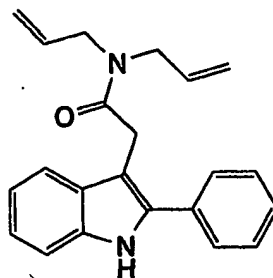
- 10 Preparation of 2-[2-(2-Chloro-phenyl)-1H-indol-3-yl]-N-isopropyl-N-pyridin-4-ylmethyl-acetamide



- 15 The above-titled compound was prepared according to the procedures of Example 2, Steps B-D using 2-chlorophenyl boronic acid in place of *ortho*-tolyl boronic acid. ES mass spectrum m/e 418 ($M+1$).

EXAMPLE 20

- 20 Preparation of N,N-Diallyl-2-(2-phenyl-1H-indol-3-yl)-acetamide

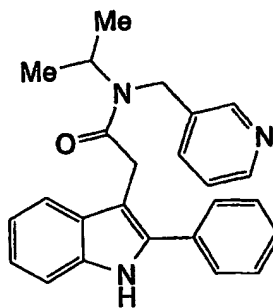


5 The above-titled compound was prepared according to the procedure of Example 2, Step D, using diallylamine in place of N-(pyridin-4-ylmethyl)propan-2-amine and 2-phenylindole-3-acetic acid in place of (2-*ortho*-tolyl-1H-indol-3-yl)-acetic acid. ES mass spectrum m/e 331 ($M+1$).

EXAMPLE 21

10

Preparation of N-Isopropyl-2-(2-phenyl-1H-indol-3-yl)-N-pyridin-3-ylmethyl-acetamide



15

Step A: Preparation of N-(pyridin-3-ylmethyl)propan-2-amine

The above-titled compound was prepared according to the procedure of Example 1, Step A using pyridine-3-carboxaldehyde in place of pyridine-4-carboxaldehyde.

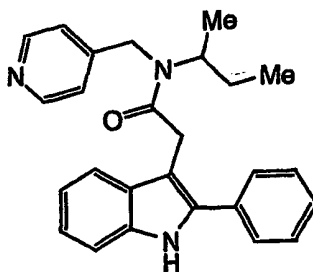
20

Step B: Preparation of N-isopropyl-2-(2-phenyl-1H-indol-3-yl)-N-pyridin-3-ylmethyl-acetamide

The above-titled compound was prepared according to the procedure of Example 2, Step D, using N-(pyridin-3-ylmethyl)propan-2-amine in place of N-(pyridin-4-ylmethyl)propan-2-amine and 2-phenylindole-3-acetic acid in place of (2-*ortho*-tolyl-1H-indol-3-yl)-acetic acid. ES mass spectrum m/e 384 (M+1).

EXAMPLE 22

10 Preparation of (S)-N-sec-Butyl-2-(2-phenyl-1H-indol-3-yl)-N-pyridin-4-ylmethyl-acetamide



15 **Step A:** Preparation of (S)-N-(pyridin-3-ylmethyl)sec-butyl-2-amine
The above-titled compound was prepared according to the procedure of Example 1, Step A using (S)-N-sec-butylamine in place of isopropylamine.

20 **Step B:** Preparation of (S)-N-sec-Butyl-2-(2-phenyl-1H-indol-3-yl)-N-pyridin-4-ylmethyl-acetamide

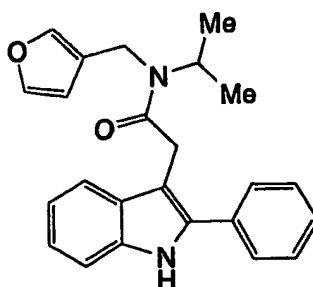
The above-titled compound was prepared according to the procedure of Example 2, Step D using (S)-N-(pyridin-3-ylmethyl)sec-butyl-2-amine, as described above in Step A, in place of N-(pyridin-4-ylmethyl)propan-2-amine and 2-phenylindole-3-acetic acid in place of (2-*ortho*-tolyl-1H-indol-3-yl)-acetic acid.

25 ES mass spectrum m/e 398 (M+1).

EXAMPLE 23

Preparation of N-Furan-3-ylmethyl-N-isopropyl-2-(2-phenyl-1H-indol-3-yl)-
acetamide

5



Step A: Preparation of N-furan-3-ylmethyl-N-isopropylamine

10 The above-titled compound was prepared according to the procedure
of Example 1, Step A using 3-furaldehyde in place of pyridine-4-carboxaldehyde.

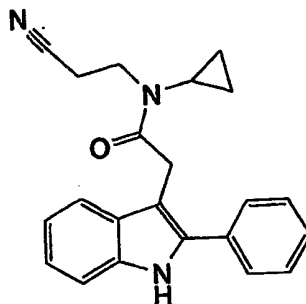
Step B: Preparation of N-furan-3-ylmethyl-N-isopropyl-2-(2-phenyl-1H-indol-
3-yl)-acetamide

15 The above-titled compound was prepared according to the
procedure of Example 2, Step D using N-furan-3-ylmethyl-N-isopropylamine, as
described above in Step A, in place of N-(pyridin-4-ylmethyl)propan-2-amine and
2-phenylindole-3-acetic acid in place of (2-*ortho*-tolyl-1H-indol-3-yl)-acetic acid.
Exact mass calcd for C₂₄H₂₄N₂O₂: 373.1911; found: 373.1928 (FAB).

20

EXAMPLE 24

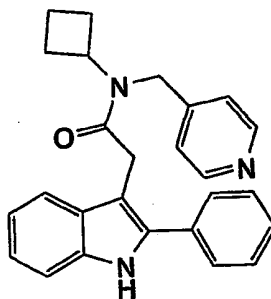
Preparation of N-(2-Cyano-ethyl)-N-cyclopropyl-2-(2-phenyl-1H-indol-3-yl)-
acetamide



- The above-titled compound was prepared according to the procedure of Example 2, Step D using 1-(cyclopropylamino)-propionitrile in place of N-(pyridin-4-ylmethyl)propan-2-amine and 2-phenylindole-3-acetic acid in place of (2-*ortho*-tolyl-1H-indol-3-yl)-acetic acid. ES mass spectrum m/e 344 (M+1).

EXAMPLE 25

- 10 Preparation of N-Cyclobutyl-2-(2-phenyl-1H-indol-3-yl)-N-pyridin-4-ylmethyl-acetamide
-

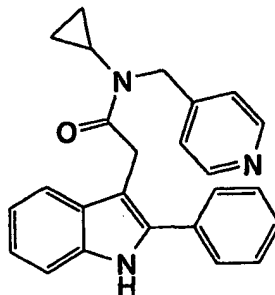


- 15 The above-titled compound was prepared according to the procedures of Example 1, Steps A and B, using cyclobutylamine in place of isopropylamine. ES mass spectrum m/e 396 (M+1).

EXAMPLE 26

Preparation of N-Cyclopropyl-2-(2-phenyl-1H-indol-3-yl)-N-pyridin-4-ylmethyl-
acetamide

5



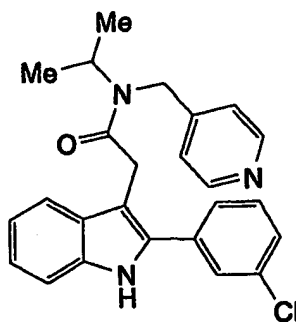
The above-titled compound was prepared according to the procedures of Example 1, Steps A and B, using cyclopropylamine in place of isopropylamine.

10 Exact mass calcd for $C_{25}H_{23}N_3O_2$: 382.1914; found: 382.1893 (FAB).

EXAMPLE 27

Preparation of 2-[2-(3-Chloro-phenyl)-1H-indol-3-yl]-N-isopropyl-N-pyridin-4-
ylmethyl-acetamide

15

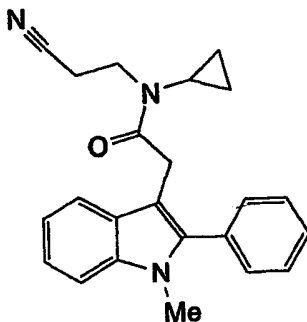


The above-titled compound was prepared according to the procedures of Example 2, Steps B-D using 3-chlorophenyl boronic acid in place of *ortho*-tolyl boronic acid. ES mass spectrum m/e 418 (M+1).

5

EXAMPLE 28

Preparation of N-(2-Cyano-ethyl)-N-cyclopropyl-2-(1-methyl-2-phenyl-1H-indol-3-yl)-acetamide



10

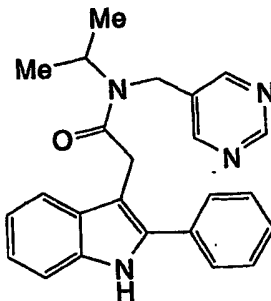
The above-titled compound was prepared according to the procedure of Example 1, Step C using N-(2-Cyano-ethyl)-N-cyclopropyl-2-(2-phenyl-1H-indol-3-yl)-acetamide (Example 24) in place of N-isopropyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide. ES mass spectrum m/e 358 (M+1).

15

EXAMPLE 29

Preparation of N-Isopropyl-2-(2-phenyl-1H-indol-3-yl)-N-pyrimidin-5-ylmethyl-acetamide

20



Step A: Preparation of N-pyrimidine-5-ylmethyl-N-isopropylamine

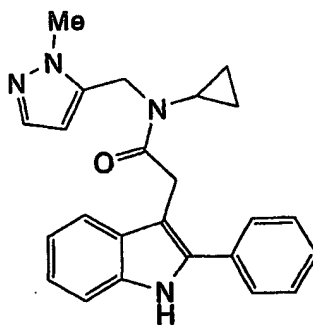
The above-titled compound was prepared according to the procedure of Example 1, Step A using pyrimidine-5-carboxaldehyde in place of pyridine-4-carboxaldehyde.

Step B: Preparation of N-Isopropyl-2-(2-phenyl-1H-indol-3-yl)-N-pyrimidin-5-ylmethyl-acetamide

The above-titled compound was prepared according to the procedure of Example 2, Step D using N-pyrimidine-5-ylmethyl-N-isopropylamine in place of N-(pyridin-4-ylmethyl)propan-2-amine and 2-phenylindole-3-acetic acid in place of (2-*ortho*-tolyl-1H-indol-3-yl)-acetic acid. Exact mass calcd for C₂₄H₂₄N₄O: 385.2023; found: 385.2040 (FAB).

EXAMPLE 30

Preparation of N-Cyclopropyl-N-(2-methyl-2H-pyrazol-3-ylmethyl)-2-(2-phenyl-1H-indol-3-yl)-acetamide



Step A: Preparation of N-Cyclopropyl-N-(2-methyl-2H-pyrazol-3-ylmethyl)amine

The above-titled compound was prepared according to the procedure of Example 1, Step A using 1-methyl-1H-pyrazole-5-carboxaldehyde in place of pyridine-4-carboxaldehyde and cyclopropylamine in place of isopropylamine.

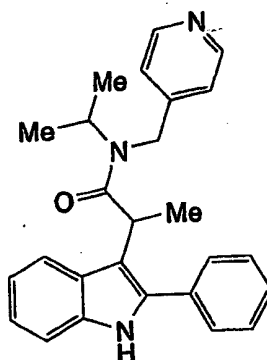
Step B: Preparation of N-Cyclopropyl-N-(2-methyl-2H-pyrazol-3-ylmethyl)-2-(2-phenyl-1H-indol-3-yl)-acetamide

The above-titled compound was prepared according to the procedure of Example 2, Step D using N-Cyclopropyl-N-(2-methyl-2H-pyrazol-3-ylmethyl) amine in place of N-(pyridin-4-ylmethyl)propan-2-amine and 2-phenylindole-3-acetic acid in place of (2-*ortho*-tolyl-1H-indol-3-yl)-acetic acid. Exact mass calcd for C₂₄H₂₄N₄O: 385.2023; found: 385.2023 (FAB).

EXAMPLE 31

10

Preparation of (+)-N-isopropyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl) propanamide



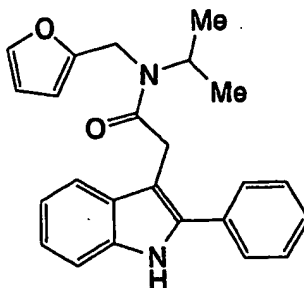
15

The above-titled compound was prepared according to the procedures of Example 3 using 2-methyl-4-oxo-4-phenylbutanoic acid in place of 4-oxo-4-phenylbutanoic acid in Step A and phenylhydrazine hydrochloride in place of *ortho*-tolylhydrazine hydrochloride in Step B. The (+)-enantiomer was obtained in optically pure form by chiral HPLC. Exact mass calcd for C₂₆H₂₇N₃O: 398.2227; found: 398.2228 (FAB).

20

EXAMPLE 32

25 Preparation of N-(2-furylmethyl)-N-isopropyl-2-(2-phenyl-1H-indol-3-yl)acetamide



Step A: Preparation of N-Furan-2-ylmethyl-N-isopropylamine

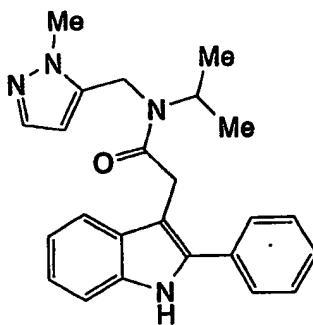
The above-titled compound was prepared according to the procedure of Example 1, Step A using 3-furaldehyde in place of pyridine-4-carboxaldehyde.

Step B: Preparation of N-(2-furymethyl)-N-isopropyl-2-(2-phenyl-1H-indol-3-yl)acetamide

The above-titled compound was prepared according to the procedure of Example 2, Step D using N-Furan-2-ylmethyl-N-isopropylamine in place of N-(pyridin-4-ylmethyl)propan-2-amine and 2-phenylindole-3-acetic acid in place of (2-*ortho*-tolyl-1H-indol-3-yl)-acetic acid. ES mass spectrum m/e 373 (M+1).

EXAMPLE 33

Preparation of N-isopropyl-N-(2-methyl-2H-pyrazol-3-ylmethyl)-2-(2-phenyl-1H-indol-3-yl)-acetamide



Step A: Preparation of N-isopropyl-N-(2-methyl-2H-pyrazol-3-ylmethyl)amine

The above-titled compound was prepared according to the procedure of Example 1, Step A using 1-methyl-1H-pyrazole-5-carboxaldehyde in place of pyridine-4-carboxaldehyde.

5

Step B: Preparation of N-isopropyl-N-(2-methyl-2H-pyrazol-3-ylmethyl)-2-(2-phenyl-1H-indol-3-yl)-acetamide

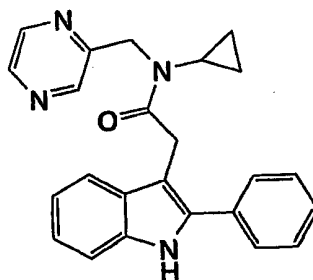
The above-titled compound was prepared according to the procedure of Example 2, Step D using N-isopropyl-N-(2-methyl-2H-pyrazol-3-ylmethyl)amine in place of N-(pyridin-4-ylmethyl)propan-2-amine and 2-phenylindole-3-acetic acid in place of (2-*ortho*-tolyl-1H-indol-3-yl)-acetic acid. ES mass spectrum m/e 387 (M+1)

10

EXAMPLE 34

15

N-cyclopropyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyrazin-2-ylmethyl)acetamide



Step A: Preparation of N-cyclopropyl-N-(pyrazin-2-ylmethyl)amine

The above-titled compound was prepared according to the procedure of Example 1, Step A using pyrazine-2-carboxaldehyde in place of pyridine-4-carboxaldehyde and cyclopropylamine in place of isopropylamine.

Step B: Preparation of N-cyclopropyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyrazin-2-ylmethyl)acetamide

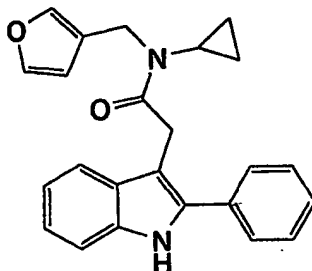
The above-titled compound was prepared according to the procedure of Example 2, Step D using N-cyclopropyl-N-(pyrazin-2-ylmethyl)amine in place of

N-(pyridin-4-ylmethyl)propan-2-amine and 2-phenylindole-3-acetic acid in place of (2-*ortho*-tolyl-1H-indol-3-yl)-acetic acid. Exact mass calcd for C₂₄H₂₂N₄O: 383.1869; found: 383.1866 (FAB).

5

EXAMPLE 35

Preparation of N-cyclopropyl-N-(3-furylmethyl)-2-(2-phenyl-1H-indol-3-yl)
acetamide



10

Step A: Preparation of N-Furan-3-ylmethyl-N-cyclopropylamine

The above-titled compound was prepared according to the procedure of Example 1, Step A using 3-furaldehyde in place of pyridine-4-carboxaldehyde and cyclopropylamine in place of isopropylamine.

15

Step B: Preparation of N-cyclopropyl-N-(3-furylmethyl)-2-(2-phenyl-1H-indol-3-yl)acetamide

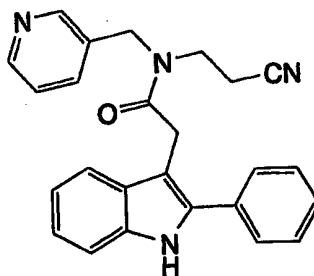
The above-titled compound was prepared according to the procedure of Example 2, Step D using N-Furan-3-ylmethyl-N-cyclopropylamine in place of N-(pyridin-4-ylmethyl)propan-2-amine and 2-phenylindole-3-acetic acid in place of (2-*ortho*-tolyl-1H-indol-3-yl)-acetic acid. Exact mass calcd for C₂₄H₂₂N₂O₂: 371.1744; found: 371.1754 (FAB).

20

25

EXAMPLE 36

Preparation of N-(2-cyanoethyl)-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-3-ylmethyl)
acetamide

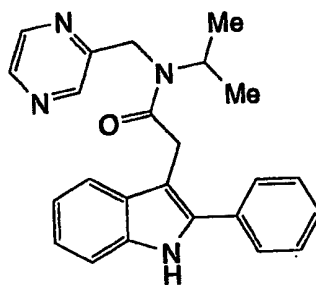


5 The above-titled compound was prepared according to the procedure of Example 2, Step D using N-(2-cyanoethyl)-N-(pyridin-3-ylmethyl)amine in place of N-(pyridin-4-ylmethyl)propan-2-amine and 2-phenylindole-3-acetic acid in place of (2-*ortho*-tolyl-1H-indol-3-yl)-acetic acid. ES mass spectrum m/e 395 (M+1)

EXAMPLE 37

10

Preparation of N-isopropyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyrazin-2-ylmethyl)acetamide



15

Step A: Preparation of N-isopropyl-N-(pyrazin-2-ylmethyl)amine

The above-titled compound was prepared according to the procedure of Example 1, Step A using pyrazine-2-carboxaldehyde in place of pyridine-4-carboxaldehyde.

20

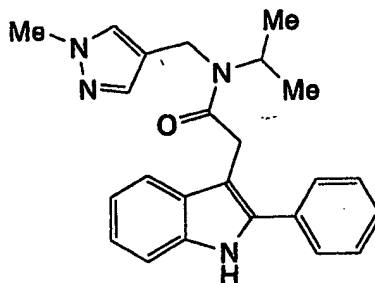
Step B: Preparation of N-isopropyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyrazin-2-ylmethyl)acetamide

The above-titled compound was prepared according to the procedure of Example 2, Step D using N-isopropyl-N-(pyrazin-2-ylmethyl)amine in place of N-(pyridin-4-ylmethyl)propan-2-amine and 2-phenylindole-3-acetic acid in place of (2-*ortho*-tolyl-1H-indol-3-yl)-acetic acid. Exact mass calcd for C₂₄H₂₄N₄O:

5 385.2035; found: 385.2023 (FAB).

EXAMPLE 38

10 N-isopropyl-N-[(1-methyl-1H-pyrazol-4-yl)methyl]-2-(2-phenyl-1H-indol-3-yl)
acetamide



15 Step A: Preparation of N-isopropyl-N-(1-methyl-1H-pyrazol-4-ylmethyl)amine
The above-titled compound was prepared according to the procedure of Example 1, Step A using 1-methyl-1H-pyrazole-4-carboxaldehyde in place of pyridine-4-carboxaldehyde.

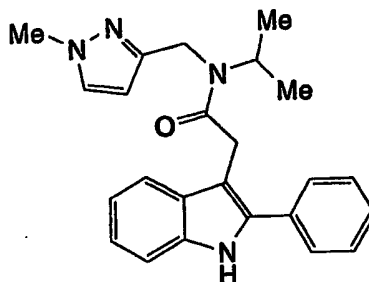
20 Step B: Preparation of N-isopropyl-N-(1-methyl-1H-pyrazol-4-ylmethyl)-2-(2-phenyl-1H-indol-3-yl)-acetamide

The above-titled compound was prepared according to the procedure of Example 2, Step D using N-isopropyl-N-(1-methyl-1H-pyrazol-4-ylmethyl)amine in place of N-(pyridin-4-ylmethyl)propan-2-amine and 2-phenylindole-3-acetic acid in place of (2-*ortho*-tolyl-1H-indol-3-yl)-acetic acid. Exact mass calcd for
25 C₂₄H₂₆N₄O: 387.2174; found: 387.2179 (FAB).

EXAMPLE 39

N-isopropyl-N-[(1-methyl-1H-pyrazol-3-yl)methyl]-2-(2-phenyl-1H-indol-3-yl)
acetamide

5



Step A: Preparation of N-isopropyl-N-(1-methyl-1H-pyrazol-3-ylmethyl)amine

10 The above-titled compound was prepared according to the procedure of Example 1, Step A using 1-methyl-1H-pyrazole-3-carboxaldehyde in place of pyridine-4-carboxaldehyde.

Step B: Preparation of N-isopropyl-N-(1-methyl-1H-pyrazol-3-ylmethyl)-2-(2-phenyl-1H-indol-3-yl)-acetamide

15

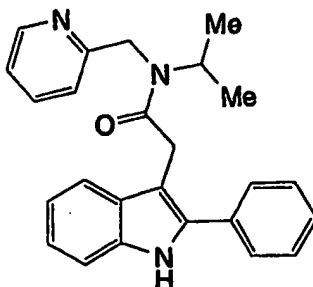
The above-titled compound was prepared according to the procedure of Example 2, Step D using N-isopropyl-N-(1-methyl-1H-pyrazol-3-ylmethyl)amine in place of N-(pyridin-4-ylmethyl)propan-2-amine and 2-phenylindole-3-acetic acid in place of (2-*ortho*-tolyl-1H-indol-3-yl)-acetic acid. Exact mass calcd for C₂₄H₂₆N₄O: 387.2156; found: 387.2179 (FAB).

20

EXAMPLE 40

Preparation of N-isopropyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-2-ylmethyl)
acetamide

25



Step A: Preparation of N-(pyridin-2-ylmethyl)propan-2-amine

5 The above-titled compound was prepared according to the procedure of Example 1, Step A using pyridine-2-carboxaldehyde in place of pyridine-4-carboxaldehyde.

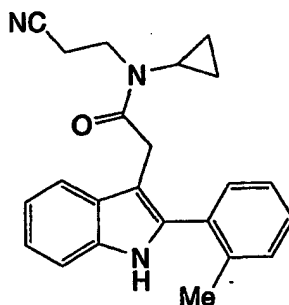
Step B: Preparation of N-isopropyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-2-ylmethyl)acetamide

10 The above-titled compound was prepared according to the procedure of Example 2, Step D N-(pyridin-2-ylmethyl)propan-2-amine in place of N-(pyridin-4-ylmethyl)propan-2-amine and 2-phenylindole-3-acetic acid in place of (2-*ortho*-tolyl-1H-indol-3-yl)-acetic acid. ES mass spectrum m/e 384 (M+1)

15

EXAMPLE 41

Preparation of N-(2-cyanoethyl)-N-cyclopropyl-2-[2-(2-methylphenyl)-1H-indol-3-yl]acetamide



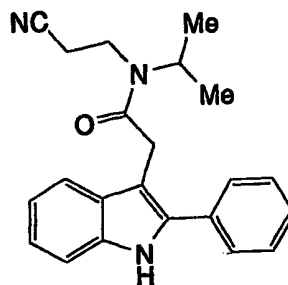
20

The above-titled compound was prepared according to the procedure of Example 2, Step D using 1-(cyclopropylamino)-propionitrile in place of N-(pyridin-4-ylmethyl)propan-2-amine. ES mass spectrum m/e 344 (M+1)

5

EXAMPLE 42

Preparation of N-(2-cyanoethyl)-N-isopropyl-2-(2-phenyl-1H-indol-3-yl)acetamide



10

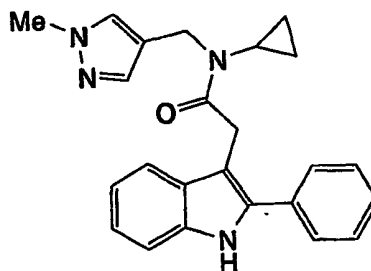
The above-titled compound was prepared according to the procedure of Example 2, Step D, using using 1-(isopropylamino)-propionitrile in place of N-(pyridin-4-ylmethyl)propan-2-amine and 2-phenylindole-3-acetic acid in place of (2-*ortho*-tolyl-1H-indol-3-yl)-acetic acid. ES mass spectrum m/e 346 (M+1)

15

EXAMPLE 43

Preparation of N-cyclopropyl-N-[(1-methyl-1H-pyrazol-4-yl)methyl]-2-(2-phenyl-1H-indol-3-yl) acetamide

20



Step A: Preparation of N-cyclopropyl-N-(1-methyl-1H-pyrazol-4-ylmethyl)
amine

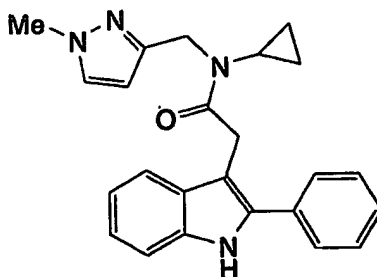
The above-titled compound was prepared according to the procedure of Example 1, Step A using 1-methyl-1H-pyrazole-4-carboxaldehyde in place of pyridine-4-carboxaldehyde and cyclopropylamine in place of isopropylamine.

Step B: Preparation of N-cyclopropyl-N-(1-methyl-1H-pyrazol-4-ylmethyl)-2-(2-phenyl-1H-indol-3-yl)-acetamide

The above-titled compound was prepared according to the procedure of Example 2, Step D using N-cyclopropyl-N-(1-methyl-1H-pyrazol-4-ylmethyl)amine in place of N-(pyridin-4-ylmethyl)propan-2-amine and 2-phenylindole-3-acetic acid in place of (2-*ortho*-tolyl-1H-indol-3-yl)-acetic acid. Exact mass calcd for C₂₄H₂₄N₄O: 385.2031; found: 385.2023 (FAB).

EXAMPLE 44

Preparation of N-cyclopropyl-N-[(1-methyl-1H-pyrazol-3-yl)methyl]-2-(2-phenyl-1H-indol-3-yl) acetamide



Step A: Preparation of N-cyclopropyl-N-(1-methyl-1H-pyrazol-3-ylmethyl)
amine

The above-titled compound was prepared according to the procedure of Example 1, Step A using 1-methyl-1H-pyrazole-3-carboxaldehyde in place of pyridine-4-carboxaldehyde and cyclopropylamine in place of isopropylamine.

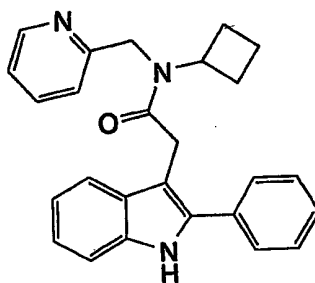
Step B: Preparation of N-cyclopropyl-N-(1-methyl-1H-pyrazol-3-ylmethyl)-
2-(2-phenyl-1H-indol-3-yl)-acetamide

The above-titled compound was prepared according to the procedure of Example 2, Step D using N-cyclopropyl-N-(1-methyl-1H-pyrazol-3-ylmethyl) amine in place of N-(pyridin-4-ylmethyl)propan-2-amine and 2-phenylindole-3-acetic acid in place of (2-*ortho*-tolyl-1H-indol-3-yl)-acetic acid. Exact mass calcd for C₂₄H₂₄N₄O: 385.2028; found: 385.2023 (FAB).

EXAMPLE 45

10

Preparation of N-cyclobutyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-2-ylmethyl)
acetamide



15

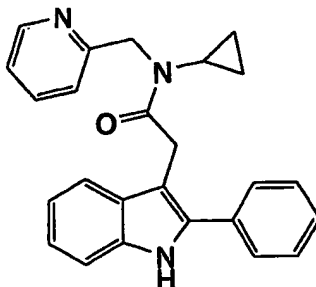
The above-titled compound was prepared according to the procedures of Example 1, Steps A and B, using pyridine-2-carboxaldehyde in place of pyridine-4-carboxaldehyde and cyclobutylamine in place of isopropylamine. Exact mass calcd for C₂₆H₂₅N₃O: 396.2066; found: 396.2070 (FAB).

20

EXAMPLE 46

Preparation of N-cyclopropyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-2-ylmethyl)
acetamide

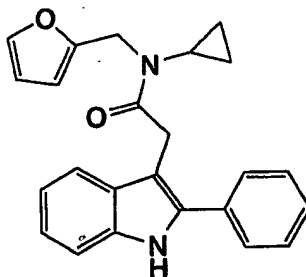
25



- The above-titled compound was prepared according to the procedures of Example 1, Steps A and B, using pyridine-2-carboxaldehyde in place of pyridine-4-carboxaldehyde and cyclopropylamine in place of isopropylamine. Exact mass calcd for $C_{25}H_{23}N_3O$: 382.1903; found: 382.1914 (FAB).

EXAMPLE 47

- 10 Preparation of N-cyclopropyl-N-(2-furylmethyl)-2-(2-phenyl-1H-indol-3-yl)acetamide



- 15 Step A: Preparation of N-Furan-2-ylmethyl-N-cyclopropylamine
The above-titled compound was prepared according to the procedure of Example 1, Step A using 2-furaldehyde in place of pyridine-4-carboxaldehyde and cyclopropylamine in place of isopropylamine.
- 20 Step B: Preparation of N-cyclopropyl-N-(2-furylmethyl)-2-(2-phenyl-1H-indol-3-yl)acetamide

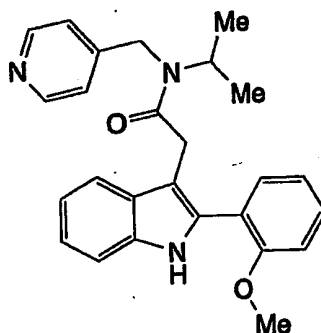
The above-titled compound was prepared according to the procedure of Example 2, Step D using N-Furan-2-ylmethyl-N-cyclopropylamine in place of N-(pyridin-4-ylmethyl)propan-2-amine and 2-phenylindole-3-acetic acid in place of (2-*ortho*-tolyl-1H-indol-3-yl)-acetic acid. ES mass spectrum m/e 317 (M+1)

5

EXAMPLE 48

Preparation of N-isopropyl-2-[2-(2-methoxyphenyl)-1H-indol-3-yl]-N-(pyridin-4-ylmethyl) acetamide

10



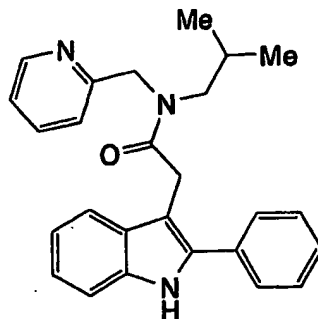
The above-titled compound was prepared according to the procedures of Example 2, Steps B-D using 2-methoxyphenyl boronic acid in place of *ortho*-tolyl boronic acid. ES mass spectrum m/e 414 (M+1)

15

EXAMPLE 49

Preparation of N-isobutyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-2-ylmethyl) acetamide

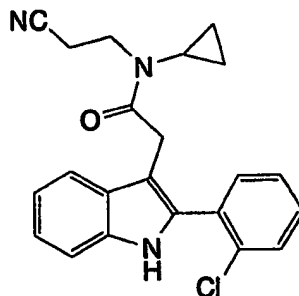
20



- 5 The above-titled compound was prepared according to the procedures of Example 1, Steps A and B, using pyridine-2-carboxaldehyde in place of pyridine-4-carboxaldehyde and isobutylamine in place of isopropylamine. Exact mass calcd for $C_{26}H_{27}N_3O$: 398.2218; found: 398.2227 (FAB).

EXAMPLE 50

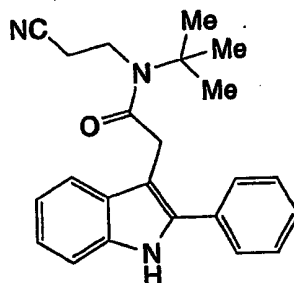
- 10 Preparation of 2-[2-(2-chlorophenyl)-1H-indol-3-yl]-N-(2-cyanoethyl)-N-cyclopropylacetamide
-



- 15 The above-titled compound was prepared according to the procedures of Example 2, Steps B-D using 2-chlorophenyl boronic acid in place of *ortho*-tolyl boronic acid and using 1-(cyclopropylamino)-propionitrile in place of N-(pyridin-4-ylmethyl)propan-2-amine. ES mass spectrum m/e 378 ($M+1$)

EXAMPLE 51

Preparation of N-(tert-butyl)-N-(2-cyanoethyl)-2-(2-phenyl-1H-indol-3-yl)acetamide



5

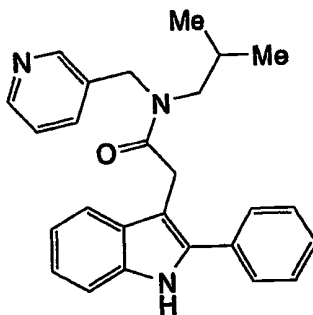
The above-titled compound was prepared according to the procedure of Example 2, Step D using 1-(*tert*-butylamino)-propionitrile in place of N-(pyridin-4-ylmethyl)propan-2-amine and 2-phenylindole-3-acetic acid in place of (2-*ortho*-tolyl-1H-indol-3-yl)-acetic acid. ES mass spectrum m/e 360 (M+1)

10

EXAMPLE 52

Preparation of N-isobutyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-3-ylmethyl)acetamide

15



The above-titled compound was prepared according to the procedures of Example 1, Steps A and B, using pyridine-3-carboxaldehyde in place of pyridine-

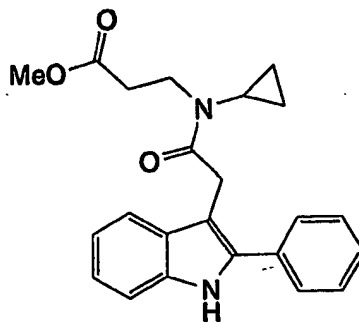
20

4-carboxaldehyde and isobutylamine in place of isopropylamine. Exact mass calcd for $C_{26}H_{27}N_3O$: 398.2242; found: 398.2227 (FAB).

EXAMPLE 53

5

Preparation of Methyl N-cyclopropyl-N-[(2-phenyl-1H-indol-3-yl)acetyl]-beta-alaninate



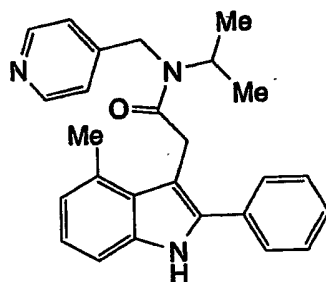
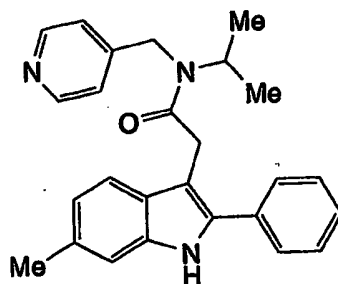
10

A solution of N-(2-Cyano-ethyl)-N-cyclopropyl-2-(2-phenyl-1H-indol-3-yl)-acetamide (0.100 g, 0.291 mmol) in MeOH : conc. HCl (5:1) was heated to reflux for 16 hours. The reaction was cooled to room temperature and poured into CH_2Cl_2 , washed with saturated aqueous $NaHCO_3$ and water, and then dried (MgSO₄), filtered, and concentrated *in vacuo*. The material was then purified by reverse phase HPLC to yield the titled compound. ES mass spectrum m/e 377 (M+1).

15

EXAMPLE 54

20 Preparation of N-isopropyl-2-(6-methyl-2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide and N-isopropyl-2-(4-methyl-2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl) acetamide

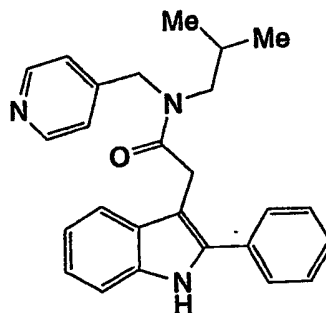


5 The above-titled compounds were prepared according to the procedure of Example 3, Step B using *meta*-tolylhydrazine hydrochloride in place of *ortho*-tolylhydrazine hydrochloride. The titled compounds were isolated as an inseparable mixture of regioisomers. Exact mass calcd for C₂₆H₂₇N₃O: 398.2227; found: 398.2213 (FAB).

10

EXAMPLE 55

Preparation of N-isobutyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)
acetamide



15

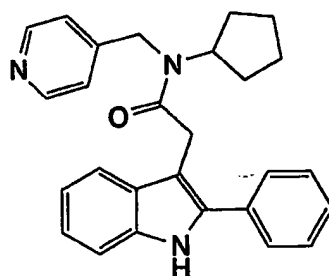
The above-titled compound was prepared according to the procedures of Example 1, Steps A and B, using isobutylamine in place of isopropylamine. Exact mass calcd for $C_{26}H_{27}N_3O$: 398.2247; found: 398.2227 (FAB).

5

EXAMPLE 56

Preparation of N-cyclopentyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl) acetamide

10



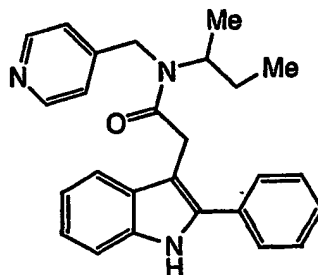
The above-titled compound was prepared according to the procedures of Example 1, Steps A and B, using cyclopentylamine in place of isopropylamine. Exact mass calcd for $C_{27}H_{27}N_3O$: 410.2258; found: 410.2227 (FAB).

15

EXAMPLE 57

Preparation of N-[(1R)-1-methylpropyl]-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl) acetamide

20



Step A: Preparation of (R)-N-(pyridin-3-ylmethyl)sec-butyl-2-amine

The above-titled compound was prepared according to the procedure of Example 1, Step A using (R)-N-sec-butylamine in place of isopropylamine.

5

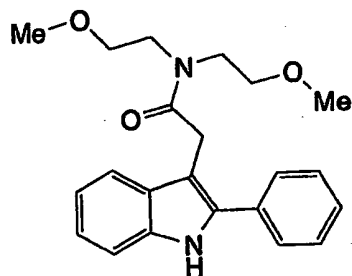
Step B: Preparation of N-[(1R)-1-methylpropyl]-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl) acetamide

The above-titled compound was prepared according to the procedure of Example 2, Step D using (R)-N-(pyridin-3-ylmethyl)sec-butyl-2-amine in place of N-(pyridin-4-ylmethyl)propan-2-amine and 2-phenylindole-3-acetic acid in place of (2-ortho-tolyl-1H-indol-3-yl)-acetic acid. ES mass spectrum m/e 398 (M+1)

10

EXAMPLE 58

15 Preparation of N,N-bis(2-methoxyethyl)-2-(2-phenyl-1H-indol-3-yl)acetamide



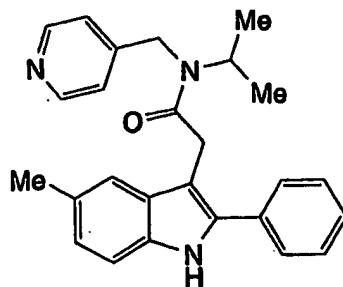
The above-titled compound was prepared according to the procedure of Example 2, Step D using 2-methoxy-N-(2-methoxyethyl) ethanamine in place of N-(pyridin-4-ylmethyl)propan-2-amine and 2-phenylindole-3-acetic acid in place of (2-ortho-tolyl-1H-indol-3-yl)-acetic acid. ES mass spectrum m/e 367 (M+1)

20

EXAMPLE 59

25

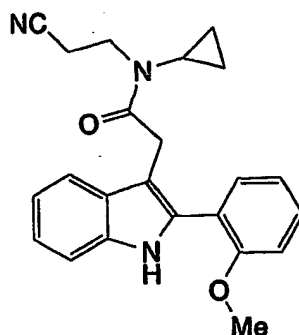
Preparation of N-isopropyl-2-(5-methyl-2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide



5 The above-titled compound was prepared according to the procedure of Example 3, Step B using *para*-tolylhydrazine hydrochloride in place of *ortho*-tolylhydrazine hydrochloride. Exact mass calcd for C₂₆H₂₇N₃O: 398.2227; found: 398.2218 (FAB).

EXAMPLE 60

10 Preparation of N-(2-cyanoethyl)-N-cyclopropyl-2-[2-(2-methoxyphenyl)-1H-indol-3-yl]acetamide

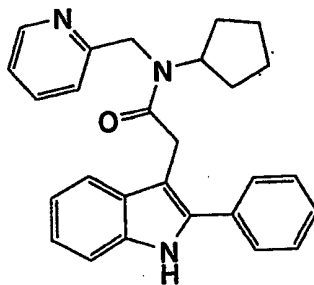


15 The above-titled compound was prepared according to the procedures of Example 2, Steps B-D using 2-methoxyphenyl boronic acid in place of *ortho*-tolyl boronic acid and using 1-(cyclopropylamino)-propionitrile in place of N-(pyridin-4-ylmethyl)propan-2-amine. ES mass spectrum m/e 374 (M+1)

EXAMPLE 61

Preparation of N-cyclopentyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-2-ylmethyl)
acetamide

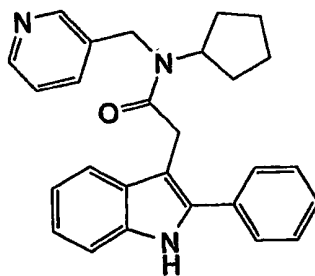
5



The above-titled compound was prepared according to the procedures
of Example 1, Steps A and B, using pyridine-2-carboxaldehyde in place of pyridine-
10 4-carboxaldehyde and cyclopentylamine in place of isopropylamine. Exact mass
calcd for C₂₇H₂₇N₃O: 410.2238; found: 410.2227 (FAB).

EXAMPLE 62

15 Preparation of N-cyclopentyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-3-ylmethyl)
acetamide



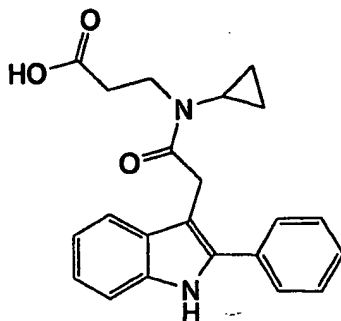
20 The above-titled compound was prepared according to the procedures
of Example 1, Steps A and B, using pyridine-3-carboxaldehyde in place of pyridine-

4-carboxaldehyde and cyclopentylamine in place of isopropylamine. Exact mass calcd for $C_{27}H_{27}N_3O$: 410.2254; found: 410.2227 (FAB).

EXAMPLE 63

5

Preparation of N-cyclopropyl-N-[(2-phenyl-1H-indol-3-yl)acetyl]-beta-alanine



10

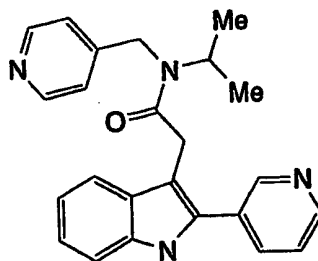
A solution of N-(2-cyano-ethyl)-N-cyclopropyl-2-(2-phenyl-1H-indol-3-yl)-acetamide (0.050 g, 0.146 mmol) in 40% aqueous NaOH (1ml) was heated to reflux for 5 hours and then cooled to room temperature. The reaction was poured into CH_2Cl_2 and washed with 3 N HCl. The aqueous solution was then washed with CH_2Cl_2 (3 x). The organic solutions were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by reverse phase HPLC provided the titled product. ES mass spectrum m/e 363 ($M+1$).

15

EXAMPLE 64

20

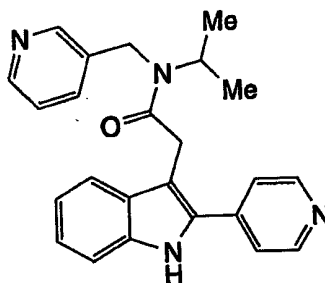
Preparation of N-Isopropyl-2-(2-pyridin-3-yl-1H-indol-3-yl)-N-pyridin-4-ylmethyl-acetamide



The above-titled compound was prepared according to the procedures of Example 2, Steps B-D using 3-pyridine boronic acid in place of *ortho*-tolyl boronic acid. ES mass spectrum m/e 385 ($M+1$).

EXAMPLE 65

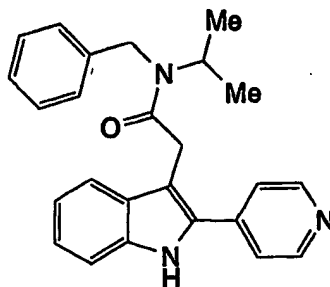
Preparation of N-Isopropyl-2-(2-pyridin-4-yl-1H-indol-3-yl)-N-pyridin-3-ylmethyl-
acetamide



The above-titled compound was prepared according to the procedures of Example 2, Steps B-D using 4-pyridine boronic acid in place of *ortho*-tolyl boronic acid and using N-(pyridin-3-ylmethyl)propan-2-amine in place of N-(pyridin-4-ylmethyl)propan-2-amine. ES mass spectrum m/e 385 ($M+1$).

EXAMPLE 66

Preparation of N-Benzyl-N-isopropyl-2-(2-pyridin-4-yl-1H-indol-3-yl)-acetamide



The above-titled compound was prepared according to the procedures of Example 2, Steps B-D using 4-pyridine boronic acid in place of *ortho*-tolyl boronic acid and using benzyl isopropylamine in place of N-(pyridin-4-ylmethyl)propan-2-amine. ES mass spectrum m/e 384 (M+1).

EXAMPLE 67

10 *In vitro* inhibition of ras farnesyl transferase

Transferase Assays. Isoprenyl-protein transferase activity assays are carried out at 30°C unless noted otherwise. A typical reaction contains (in a final volume of 50 μ L): [3 H]farnesyl diphosphate, Ras protein, 50 mM HEPES, pH 7.5, a modulating anion (for example 5mM ATP), 5 mM $MgCl_2$, 5 mM dithiothreitol, 15 10 μ M $ZnCl_2$, 0.1% polyethyleneglycol (PEG) (15,000-20,000 mw) and isoprenyl-protein transferase. The FPTase employed in the assay is prepared by recombinant expression as described in Omer, C.A., Kral, A.M., Diehl, R.E., Prendergast, G.C., Powers, S., Allen, C.M., Gibbs, J.B. and Kohl, N.E. (1993) *Biochemistry* 32:5167-5176. After thermally pre-equilibrating the assay mixture in the absence of enzyme, reactions are initiated by the addition of isoprenyl-protein transferase and stopped at timed intervals (typically 15 minutes) by the addition of 1 M HCl in ethanol (1 mL). The quenched reactions are allowed to stand for 15 minutes (to complete the precipitation process). After adding 2 mL of 100% ethanol, the reactions are vacuum-filtered through Whatman GF/C filters. Filters are washed 20 four times with 2 mL aliquots of 100% ethanol, mixed with scintillation fluid (10 mL) and then counted in a Beckman LS3801 scintillation counter.

For inhibition studies, assays are run as described above, except inhibitors are prepared as concentrated solutions in 100% dimethyl sulfoxide and then

diluted 20-fold into the enzyme assay mixture. Substrate concentrations for inhibitor IC₅₀ determinations are as follows: FTase, 650 nM Ras-CVLS (SEQ.ID.NO.: 2), 100 nM farnesyl diphosphate.

- 5 The compounds of the instant invention are tested for inhibitory activity against human FPTase by the assay described above.

EXAMPLE 68

Modified *In vitro* GGTase inhibition assay

- 10 The modified geranylgeranyl-protein transferase inhibition assay is carried out at room temperature. A typical reaction contains (in a final volume of 50 μ L): [³H]geranylgeranyl diphosphate, biotinylated Ras peptide, 50 mM HEPES, pH 7.5, a modulating anion (for example 10 mM glycerophosphate or 5mM ATP), 5 mM MgCl₂, 10 μ M ZnCl₂, 0.1% PEG (15,000-20,000 mw), 2 mM dithiothreitol,
15 and geranylgeranyl-protein transferase type I (GGTase). The GGTase-type I enzyme employed in the assay is prepared as described in U.S. Patent No. 5,470,832, incorporated by reference. The Ras peptide is derived from the K4B-Ras protein and has the following sequence: biotinyl-GKKKKKKSKTKCVIM (single amino acid code) (SEQ.ID.NO.: 2). Reactions are initiated by the addition of GGTase
20 and stopped at timed intervals (typically 15 minutes) by the addition of 200 μ L of a 3 mg/mL suspension of streptavidin SPA beads (Scintillation Proximity Assay beads, Amersham) in 0.2 M sodium phosphate, pH 4, containing 50 mM EDTA, and 0.5% BSA. The quenched reactions are allowed to stand for 2 hours before analysis on a Packard TopCount scintillation counter.

- 25 For inhibition studies, assays are run as described above, except inhibitors are prepared as concentrated solutions in 100% dimethyl sulfoxide and then diluted 25 fold into the enzyme assay mixture. IC₅₀ values are determined with Ras peptide near K_M concentrations. Enzyme and substrate concentrations for inhibitor IC₅₀ determinations are as follows: 75 pM GGTase-I, 1.6 μ M Ras peptide, 100 nM
30 geranylgeranyl diphosphate.

 The compounds of the instant invention are tested for inhibitory activity against human GGTase-type I by the assay described above.

EXAMPLE 69

Cell-based *in vitro* ras farnesylation assay

The cell line used in this assay is a v-ras line derived from either
5 Rat1 or NIH3T3 cells, which expressed viral Ha-ras p21. The assay is performed
essentially as described in DeClue, J.E. et al., Cancer Research 51:712-717, (1991).
Cells in 10 cm dishes at 50-75% confluency are treated with the test compound (final
concentration of solvent, methanol or dimethyl sulfoxide, is 0.1%). After 4 hours
at 37°C, the cells are labeled in 3 ml methionine-free DMEM supplemented with
10 10% regular DMEM, 2% fetal bovine serum and 400 µCi [³⁵S]methionine (1000
Ci/mmol). After an additional 20 hours, the cells are lysed in 1 ml lysis buffer (1%
NP40/20 mM HEPES, pH 7.5/5 mM MgCl₂/1mM DTT/10 mg/ml aprotinin/2 mg/ml
leupeptin/2 mg/ml antipain/0.5 mM PMSF) and the lysates cleared by centrifugation
at 100,000 x g for 45 minutes. Aliquots of lysates containing equal numbers of acid-
15 precipitable counts are brought to 1 ml with IP buffer (lysis buffer lacking DTT) and
immuno-precipitated with the ras-specific monoclonal antibody Y13-259 (Furth, M.E.
et al., J. Virol. 43:294-304, (1982)). Following a 2 hour antibody incubation at 4°C,
200 µl of a 25% suspension of protein A-Sepharose coated with rabbit anti rat IgG
is added for 45 minutes. The immuno-precipitates are washed four times with IP
20 buffer (20 mM HEPES, pH 7.5/1 mM EDTA/1% Triton X-100/0.5% deoxycholate/
0.1%/SDS/0.1 M NaCl) boiled in SDS-PAGE sample buffer and loaded on 13%
acrylamide gels. When the dye front reached the bottom, the gel is fixed, soaked in
Enlightening, dried and autoradiographed. The intensities of the bands corresponding
to farnesylated and nonfarnesylated ras proteins are compared to determine the
25 percent inhibition of farnesyl transfer to protein.

EXAMPLE 70

Cell-based *in vitro* growth inhibition assay

30 To determine the biological consequences of FPTase inhibition,
the effect of the compounds of the instant invention on the anchorage-independent
growth of Rat1 cells transformed with either a v-ras, v-raf, or v-mos oncogene is
tested. Cells transformed by v-Raf and v-Mos may be included in the analysis to
evaluate the specificity of instant compounds for Ras-induced cell transformation.
35 Rat 1 cells transformed with either v-ras, v-raf, or v-mos are seeded

at a density of 1×10^4 cells per plate (35 mm in diameter) in a 0.3% top agarose layer in medium A (Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum) over a bottom agarose layer (0.6%). Both layers contain 0.1% methanol or an appropriate concentration of the instant compound (dissolved in methanol at 1000 times the final concentration used in the assay). The cells are fed twice weekly with 0.5 ml of medium A containing 0.1% methanol or the concentration of the instant compound. Photomicrographs are taken 16 days after the cultures are seeded and comparisons are made.

10

EXAMPLE 71

Construction of SEAP reporter plasmid pDSE100

The SEAP reporter plasmid, pDSE100 was constructed by ligating a restriction fragment containing the SEAP coding sequence into the plasmid pCMV-RE-AKI. The SEAP gene is derived from the plasmid pSEAP2-Basic (Clontech, Palo Alto, CA). The plasmid pCMV-RE-AKI was constructed by Deborah Jones (Merck) and contains 5 sequential copies of the 'dyad symmetry response element' cloned upstream of a 'CAT-TATA' sequence derived from the cytomegalovirus immediate early promoter. The plasmid also contains a bovine growth hormone poly-A sequence.

20

The plasmid, pDSE100 was constructed as follows. A restriction fragment encoding the SEAP coding sequence was cut out of the plasmid pSEAP2-Basic using the restriction enzymes EcoR1 and HpaI. The ends of the linear DNA fragments were filled in with the Klenow fragment of *E. coli* DNA Polymerase I. The 'blunt ended' DNA containing the SEAP gene was isolated by electrophoresing the digest in an agarose gel and cutting out the 1694 base pair fragment. The vector plasmid pCMV-RE-AKI was linearized with the restriction enzyme Bgl-II and the ends filled in with Klenow DNA Polymerase I. The SEAP DNA fragment was blunt end ligated into the pCMV-RE-AKI vector and the ligation products were transformed into DH5-alpha *E. coli* cells (Gibco-BRL). Transformants were screened for the proper insert and then mapped for restriction fragment orientation. Properly oriented recombinant constructs were sequenced across the cloning junctions to verify the correct sequence. The resulting plasmid contains the SEAP coding sequence

25
30

downstream of the DSE and CAT-TATA promoter elements and upstream of the BGH poly-A sequence.

Alternative Construction of SEAP reporter plasmid, pDSE101

5 The SEAP repotrer plasmid, pDSE101 is also constructed by ligating a restriction fragment containing the SEAP coding sequence into the plasmid pCMV-RE-AKI. The SEAP gene is derived from plasmid pGEM7zf(-)/SEAP.

10 The plasmid pDSE101 was constructed as follows: A restriction fragment containing part of the SEAP gene coding sequence was cut out of the plasmid pGEM7zf(-)/SEAP using the restriction enzymes Apa I and KpnI. The ends of the linear DNA fragments were chewed back with the Klenow fragment of *E. coli* DNA Polymerase I. The "blunt ended" DNA containing the truncated SEAP gene was isolated by electrophoresing the digest in an agarose gel and cutting out the 1910 base pair fragment. This 1910 base pair fragment was ligated into the plasmid pCMV-RE-AKI which had been cut with Bgl-II and filled in with *E. coli* Klenow
15 fragment DNA polymerase. Recombinant plasmids were screened for insert orientation and sequenced through the ligated junctions. The plasmid pCMV-RE-AKI is derived from plasmid pCMVIE-AKI-DHFR (Whang, Y., Silberklang, M., Morgan, A., Munshi, S., Lenny, A.B., Ellis, R.W., and Kieff, E. (1987) *J. Virol.*, 61, 1796-
20 1807) by removing an EcoRI fragment containing the DHFR and Neomycin markers. Five copies of the fos promoter serum response element were inserted as described previously (Jones, R.E., Defeo-Jones, D., McAvoy, E.M., Vuocolo, G.A., Wegrzyn, R.J., Haskell, K.M. and Oliff, A. (1991) *Oncogene*, 6, 745-751) to create plasmid pCMV-RE-AKI.

25 The plasmid pGEM7zf(-)/SEAP was constructed as follows. The SEAP gene was PCRed, in two segments from a human placenta cDNA library (Clontech) using the following oligos.

30 Sense strand N-terminal SEAP : 5' GAGAGGGAATTCGGGCCCTTCCTGCAT
GCTGCTGCTGCTGCTGCTGCTGGGC 3' (SEQ.ID.NO.:4)

 Antisense strand N-terminal SEAP: 5' GAGAGAGCTCGAGGTTAACCCGGGT
GCGCGGCGTCGGTGGT 3' (SEQ.ID.NO.:5)

35 Sense strand C-terminal SEAP: 5' GAGAGAGTCTAGAGTTAACCCGTGGTCC

CCGCGTTGCTTCCT 3' (SEQ.ID.NO.:6)

Antisense strand C-terminal SEAP: 5' GAAGAGGAAGCTTGGTACCGCCACTG
GGCTGTAGGTGGTGGCT 3' (SEQ.ID.NO.:7)

5

The N-terminal oligos (SEQ.ID.NO.: 4 and SEQ.ID.NO.: 5) were used to generate a 1560 bp N-terminal PCR product that contained EcoRI and HpaI restriction sites at the ends. The Antisense N-terminal oligo (SEQ.ID.NO.: 5) introduces an internal translation STOP codon within the SEAP gene along with the HpaI site. The C-terminal oligos (SEQ.ID.NO.: 6 and SEQ.ID.NO.: 7) were used to amplify a 412 bp C-terminal PCR product containing HpaI and HindIII restriction sites. The sense strand C-terminal oligo (SEQ.ID.NO.: 6) introduces the internal STOP codon as well as the HpaI site. Next, the N-terminal amplicon was digested with EcoRI and HpaI while the C-terminal amplicon was digested with HpaI and HindIII. The two fragments comprising each end of the SEAP gene were isolated by electro-phoresing the digest in an agarose gel and isolating the 1560 and 412 base pair fragments. These two fragments were then co-ligated into the vector pGEM7zf (-) (Promega) which had been restriction digested with EcoRI and HindIII and isolated on an agarose gel. The resulting clone, pGEM7zf(-)/SEAP contains the coding sequence for the SEAP gene from amino acids.

Construction of a constitutively expressing SEAP plasmid pCMV-SEAP-A

An expression plasmid constitutively expressing the SEAP protein was created by placing the sequence encoding a truncated SEAP gene downstream of the cytomegalovirus (CMV) IE-1 promoter. The expression plasmid also includes the CMV intron A region 5' to the SEAP gene as well as the 3' untranslated region of the bovine growth hormone gene 3' to the SEAP gene.

The plasmid pCMVIE-AKI-DHFR (Whang, Y., Silberklang, M., Morgan, A., Munshi, S., Lenny, A.B., Ellis, R.W., and Kieff, E. (1987) J. Virol., 61:1796-1807) containing the CMV immediate early promoter was cut with EcoRI generating two fragments. The vector fragment was isolated by agarose electrophoresis and religated. The resulting plasmid is named pCMV-AKI. Next, the cytomegalovirus intron A nucleotide sequence was inserted downstream of the CMV IE1 promoter in pCMV-AKI. The intron A sequence was isolated from a genomic clone bank and subcloned into pBR322 to generate plasmid p16T-286.

The intron A sequence was mutated at nucleotide 1856 (nucleotide numbering as in Chapman, B.S., Thayer, R.M., Vincent, K.A. and Haigwood, N.L., Nuc.Acids Res. 19, 3979-3986) to remove a SacI restriction site using site directed mutagenesis. The mutated intron A sequence was PCR'd from the plasmid p16T-287 using the
5 following oligos.

Sense strand: 5' GGCAGAGCTCGTTTAGTGAACCGTCAG 3' (SEQ.ID.NO.: 8)

Antisense strand: 5' GAGAGATCTCAAGGACGGTGACTGCAG 3' (SEQ.ID.NO.:
10 9)

These two oligos generate a 991 base pair fragment with a SacI site incorporated by the sense oligo and a Bgl-II fragment incorporated by the antisense oligo. The PCR fragment is trimmed with SacI and Bgl-II and isolated on an agarose
15 gel. The vector pCMV-AKI is cut with SacI and Bgl-II and the larger vector fragment isolated by agarose gel electrophoresis. The two gel isolated fragments are ligated at their respective SacI and Bgl-II sites to create plasmid pCMV-AKI-InA.

The DNA sequence encoding the truncated SEAP gene is inserted into the pCMV-AKI-InA plasmid at the Bgl-II site of the vector. The SEAP gene is cut
20 out of plasmid pGEM7zf(-)/SEAP (described above) using EcoRI and HindIII. The fragment is filled in with Klenow DNA polymerase and the 1970 base pair fragment isolated from the vector fragment by agarose gel electrophoresis. The pCMV-AKI-InA vector is prepared by digesting with Bgl-II and filling in the ends with Klenow DNA polymerase. The final construct is generated by blunt end ligating the SEAP
25 fragment into the pCMV-AKI-InA vector. Transformants were screened for the proper insert and then mapped for restriction fragment orientation. Properly oriented recombinant constructs were sequenced across the cloning junctions to verify the correct sequence. The resulting plasmid, named pCMV-SEAP-A (deposited in the ATCC under Budapest Treaty on August 27, 1998, and designated ATCC), contains
30 a modified SEAP sequence downstream of the cytomegalovirus immediately early promoter IE-1 and intron A sequence and upstream of the bovine growth hormone poly-A sequence. The plasmid expresses SEAP in a constitutive manner when transfected into mammalian cells.

Alternative construction of a constitutively expressing SEAP plasmid pCMV-SEAP-B

An expression plasmid constitutively expressing the SEAP protein can be created by placing the sequence encoding a truncated SEAP gene downstream of the cytomegalovirus (CMV) IE-1 promoter and upstream of the 3' untranslated region of the bovine growth hormone gene.

5 The plasmid pCMVIE-AKI-DHFR (Whang, Y., Silberklang, M., Morgan, A., Munshi, S., Lenny, A.B., Ellis, R.W., and Kieff, E. (1987) J. Virol., 61:1796-1807) containing the CMV immediate early promoter and bovine growth hormone poly-A sequence can be cut with EcoRI generating two fragments. The vector fragment can be isolated by agarose electrophoresis and religated. The resulting plasmid is named pCMV-AKI. The DNA sequence encoding the truncated SEAP gene can be inserted into the pCMV-AKI plasmid at a unique Bgl-II in the vector. The SEAP gene is cut out of plasmid pGEMzf(-)/SEAP (described above) using EcoRI and HindIII. The fragments are filled in with Klenow DNA polymerase and the 1970 base pair fragment is isolated from the vector fragment by agarose gel electrophoresis. The pCMV-AKI vector is prepared by digesting with Bgl-II and filling in the ends with Klenow DNA polymerase. The final construct is generated by blunt end ligating the SEAP fragment into the vector and transforming the ligation reaction into *E. coli* DH5acells. Transformants can then be screened for the proper insert and mapped for restriction fragment orientation. Properly oriented recombinant constructs would be sequenced across the cloning junctions to verify the correct sequence. The resulting plasmid, named pCMV-SEAP-B contains a modified SEAP sequence downstream of the cytomegalovirus immediate early promoter, IE1, and upstream of a bovine growth hormone poly-A sequence. The plasmid would express SEAP in a constitutive manner when transfected into mammalian cells.

Cloning of a Myristylated viral-H-ras expression plasmid pSMS600

A DNA fragment containing viral-H-ras can be PCR'd from plasmid "HB-11 (deposited in the ATCC under Budapest Treaty on August 27, 1997, and designated ATCC 209,218) using the following oligos.

Sense strand:

5'TCTCCTCGAGGCCACCATGGGGAGTAGCAAGAGCAAGCCTAAGGACCC
CAGCCAGCGCCGGATGACAGAATACAAGCTTGTGGTGG 3'. (SEQ.ID.NO.:

35 10)

Antisense:

5'CACATCTAGATCAGGACAGCACAGACTTGCAGC 3'.

(SEQ.ID.NO.: 11)

5

A sequence encoding the first 15 aminoacids of the v-src gene, containing a myristylation site, is incorporated into the sense strand oligo. The sense strand oligo also optimizes the 'Kozak' translation initiation sequence immediately 5' to the ATG start site. To prevent prenylation at the viral-*ras* C-terminus, cysteine 186 would be mutated to a serine by substituting a G residue for a C residue in the C-terminal antisense oligo. The PCR primer oligos introduce an XhoI site at the 5' end and a XbaI site at the 3' end. The XhoI-XbaI fragment can be ligated into the mammalian expression plasmid pCI (Promega) cut with XhoI and XbaI. This results in a plasmid, pSMS600, in which the recombinant myr-viral-H-*ras* gene is constitutively transcribed from the CMV promoter of the pCI vector.

10

15

Cloning of a viral-H-*ras*-CVLL expression plasmid pSMS601

A viral-H-*ras* clone with a C-terminal sequence encoding the amino acids CVLL can be cloned from the plasmid "HB-11" by PCR using the following oligos.

20

Sense strand:

5'TCTCCTCGAGGCCACCATGACAGAATACAAGCTTGTGGTGG-3'

(SEQ.ID.NO.: 12)

25

Antisense strand:

5'CACTCTAGACTGGTGTGAGAGCAGCACACACTTGCAGC-3' (SEQ.ID.NO.:

13)

30

The sense strand oligo optimizes the 'Kozak' sequence and adds an XhoI site. The antisense strand mutates serine 189 to leucine and adds an XbaI site. The PCR fragment can be trimmed with XhoI and XbaI and ligated into the XhoI-XbaI cut vector pCI (Promega). This results in a plasmid, pSMS601, in which the mutated viral-H-*ras*-CVLL gene is constitutively transcribed from the CMV promoter of the pCI vector.

35

Cloning of cellular-H-ras-Leu61 expression plasmid pSMS620

The human cellular-H-ras gene can be PCR'd from a human cerebral cortex cDNA library (Clontech) using the following oligonucleotide primers.

5

Sense strand:

5'-GAGAGAATTCGCCACCATGACGGAATATAAGCTGGTGG-3'

(SEQ.ID.NO.: 14)

10 Antisense strand:

5'-GAGAGTCGACGCGTCAGGAGAGCACACACTTGC-3' (SEQ.ID.NO.: 15)

The primers will amplify a c-H-Ras encoding DNA fragment with the primers contributing an optimized 'Kozak' translation start sequence, an EcoRI site at the N-terminus and a Sal I site at the C-terminal end. After trimming the ends of the PCR product with EcoRI and Sal I, the c-H-ras fragment can be ligated into an EcoRI -Sal I cut mutagenesis vector pAlter-1 (Promega). Mutation of glutamine-61 to a leucine can be accomplished using the manufacturer's protocols and the following oligonucleotide:

20

5'-CCGCCGGCCTGGAGGAGTACAG-3' (SEQ.ID.NO.: 16)

After selection and sequencing for the correct nucleotide substitution, the mutated c-H-ras-Leu61 can be excised from the pAlter-1 vector, using EcoRI and Sal I, and be directly ligated into the vector pCI (Promega) which has been digested with EcoRI and Sal I. The new recombinant plasmid, pSMS620, will constitutively transcribe c-H-ras-Leu61 from the CMV promoter of the pCI vector.

25

Cloning of a c-N-ras-Val-12 expression plasmid pSMS630

The human c-N-ras gene can be PCR'd from a human cerebral cortex cDNA library (Clontech) using the following oligonucleotide primers.

30

Sense strand:

5'-GAGAGAATTCGCCACCATGACTGAGTACAAACTGGTGG-3'

35 (SEQ.ID.NO.: 17)

Antisense strand:

5'-GAGAGTCGACTTGTTACATCACCACACATGGC-3' (SEQ.ID.NO.: 18)

5 The primers will amplify a c-N-Ras encoding DNA fragment with the
primers contributing an optimized 'Kozak' translation start sequence, an EcoRI site
at the N-terminus and a Sal I site at the C-terminal end. After trimming the ends of
the PCR product with EcoRI and Sal I, the c-N-ras fragment can be ligated into an
EcoRI-Sal I cut mutagenesis vector pAlter-1 (Promega). Mutation of glycine-12 to
10 a valine can be accomplished using the manufacturer's protocols and the following
oligonucleotide:

5'-GTTGGAGCAGTTGGTGTGTTGGG-3' (SEQ.ID.NO.: 19)

15 After selection and sequencing for the correct nucleotide substitution,
the mutated c-N-ras-Val-12 can be excised from the pAlter-1 vector, using EcoRI and
Sal I, and be directly ligated into the vector pCI (Promega) which has been digested
with EcoRI and Sal I. The new recombinant plasmid, pSMS630, will constitutively
transcribe c-N-ras-Val-12 from the CMV promoter of the pCI vector.

20

Cloning of a c-K4B-ras-Val-12 expression plasmid pSMS640

The human c-K4B-ras gene can be PCR'd from a human cerebral
cortex cDNA library (Clontech) using the following oligo-nucleotide primers.

25 Sense strand:

5'-GAGAGGTACCGCCACCATGACTGAATATAAACTTGTGG-3'
(SEQ.ID.NO.: 20)

Antisense strand:

30 5'-CTCTGTCGACGTATTTACATAATTACACACTTTGTC-3' (SEQ.ID.NO.: 21)

The primers will amplify a c-K4B-Ras encoding DNA fragment with
the primers contributing an optimized 'Kozak' translation start sequence, a KpnI site
at the N-terminus and a Sal I site at the C-terminal end. After trimming the ends of

the PCR product with Kpn I and Sal I, the c-K4B-*ras* fragment can be ligated into a KpnI -Sal I cut mutagenesis vector pAlter-1 (Promega). Mutation of cysteine-12 to a valine can be accomplished using the manufacturer's protocols and the following oligonucleotide:

5

5'-GTAGTTGGAGCTGTTGGCGTAGGC-3' (SEQ.ID.NO.: 22)

After selection and sequencing for the correct nucleotide substitution, the mutated c-K4B-*ras*-Val-12 can be excised from the pAlter-1 vector, using KpnI and Sal I, and be directly ligated into the vector pCI (Promega) which has been digested with KpnI and Sal I. The new recombinant plasmid will constitutively transcribe c-K4B-*ras*-Val-12 from the CMV promoter of the pCI vector.

10

Cloning of c-K-ras4A-Val-12 expression plasmid pSMS650

15

The human c-K4A-*ras* gene can be PCR'd from a human cerebral cortex cDNA library (Clontech) using the following oligo-nucleotide primers.

Sense strand:

5'-GAGAGGTACCGCCACCATGACTGAATATAAACTTGTGG-3'

20

(SEQ.ID.NO.: 23)

Antisense strand:

5'-CTCTGTCGACAGATTACATTATAATGCATTTTTTAATTTTCACAC-3'

(SEQ.ID.NO.: 24)

25

The primers will amplify a c-K4A-Ras encoding DNA fragment with the primers contributing an optimized 'Kozak' translation start sequence, a KpnI site at the N-terminus and a Sal I site at the C-terminal end. After trimming the ends of the PCR product with Kpn I and Sal I, the c-K-ras4A fragment can be ligated into a KpnI -Sal I cut mutagenesis vector pAlter-1 (Promega). Mutation of cysteine-12 to a valine can be accomplished using the manufacturer's protocols and the following oligonucleotide:

30

5'-GTAGTTGGAGCTGTTGGCGTAGGC-3' (SEQ.ID.NO.: 25)

35

After selection and sequencing for the correct nucleotide substitution, the mutated c-K4A-*ras*-Val-12 can be excised from the pAlter-1 vector, using KpnI and Sal I, and be directly ligated into the vector pCI (Promega) which has been digested with KpnI and Sal I. The new recombinant plasmid, pSMS650, will
5 constitutively transcribe c-K4A-*ras*-Val-12 from the CMV promoter of the pCI vector.

SEAP assay

Human C33A cells (human epithelial carcinoma - ATTC collection)
10 are seeded in 10cm tissue culture plates in DMEM + 10% fetal calf serum + 1X Pen/Strep + 1X glutamine + 1X NEAA. Cells are grown at 37°C in a 5% CO₂ atmosphere until they reach 50-80% of confluency.

The transient transfection is performed by the CaPO₄ method (Sambrook et al., 1989). Thus, expression plasmids for H-*ras*, N-*ras*, K-*ras*, Myr-*ras* or H-*ras*-CVLL are co-precipitated with the DSE-SEAP reporter construct. (A
15 *ras* expression plasmid is not included when the cell is transfected with the pCMV-SEAP plasmid.) For 10cm plates 600 µl of CaCl₂-DNA solution is added dropwise while vortexing to 600 µl of 2X HBS buffer to give 1.2 ml of precipitate solution (see recipes below). This is allowed to sit at room temperature for 20 to 30 minutes.
20 While the precipitate is forming, the media on the C33A cells is replaced with DMEM (minus phenol red; Gibco cat. No. 31053-028)+ 0.5% charcoal stripped calf serum + 1X (Pen/Strep, Glutamine and nonessential aminoacids). The CaPO₄-DNA precipitate is added dropwise to the cells and the plate rocked gently to distribute. DNA uptake is allowed to proceed for 5-6 hours at 37°C under a 5% CO₂
25 atmosphere.

Following the DNA incubation period, the cells are washed with PBS and trypsinized with 1ml of 0.05% trypsin. The 1 ml of trypsinized cells is diluted into 10 ml of phenol red free DMEM + 0.2% charcoal stripped calf serum + 1X (Pen/Strep, Glutamine and NEAA). Transfected cells are plated in a 96 well micro-
30 titer plate (100 µl/well) to which drug, diluted in media, has already been added in a volume of 100 µl. The final volume per well is 200 µl with each drug concentration repeated in triplicate over a range of half-log steps.

Incubation of cells and drugs is for 36 hours at 37°C under CO₂. At the end of the incubation period, cells are examined micro-scopically for evidence of

cell distress. Next, 100 μ l of media containing the secreted alkaline phosphatase is removed from each well and transferred to a microtube array for heat treatment at 65°C for 1 hour to inactivate endogenous alkaline phosphatases (but not the heat stable secreted phosphatase).

- 5 The heat treated media is assayed for alkaline phosphatase by a luminescence assay using the luminescence reagent CSPD® (Tropix, Bedford, Mass.). A volume of 50 μ l media is combined with 200 μ l of CSPD cocktail and incubated for 60 minutes at room temperature. Luminescence is monitored using an ML2200 microplate luminometer (Dynatech). Luminescence reflects the level
- 10 of activation of the fos reporter construct stimulated by the transiently expressed protein.

DNA-CaPO₄ precipitate for 10cm. plate of cells

	Ras expression plasmid (1 μ g/ μ l)	10 μ l
15	DSE-SEAP Plasmid (1 μ g/ μ l)	2 μ l
	Sheared Calf Thymus DNA (1 μ g/ μ l)	8 μ l
	2M CaCl ₂	74 μ l
	dH ₂ O	506 μ l

- 20 2X HBS Buffer
 280mM NaCl
 10mM KCl
 1.5mM Na₂HPO₄ 2H₂O
 12mM dextrose
- 25 50mM HEPES
 Final pH = 7.05

Luminescence Buffer (26ml)

	Assay Buffer	20ml
30	Emerald Reagent™ (Tropix)	2.5ml
	100mM homoarginine	2.5ml
	CSPD Reagent® (Tropix)	1.0ml

Assay Buffer

Add 0.05M Na₂CO₃ to 0.05M NaHCO₃ to obtain pH 9.5.

Make 1mM in MgCl₂

5

EXAMPLE 72

The processing assays employed are modifications of that described by DeClue et al [Cancer Research 51, 712-717, 1991].

10 K4B-Ras processing inhibition assay

PSN-1 (human pancreatic carcinoma) or viral-K4B-ras-transformed Rat1 cells are used for analysis of protein processing. Subconfluent cells in 100 mm dishes are fed with 3.5 ml of media (methionine-free RPMI supplemented with 2% fetal bovine serum or cysteine-free/methionine-free DMEM supplemented with 0.035 ml of 200 mM glutamine (Gibco), 2% fetal bovine serum, respectively) containing the desired concentration of test compound, lovastatin or solvent alone. Cells treated with lovastatin (5-10 μ M), a compound that blocks Ras processing in cells by inhibiting a rate-limiting step in the isoprenoid biosynthetic pathway, serve as a positive control. Test compounds are prepared as 1000x concentrated solutions in DMSO to yield a final solvent concentration of 0.1%. Following incubation at 37°C for 2 hours 204 μ Ci/ml [³⁵S]Pro-Mix (Amersham, cell labeling grade) is added.

After introducing the label amino acid mixture, the cells are incubated at 37°C for an additional period of time (typically 6 to 24 hours). The media is then removed and the cells are washed once with cold PBS. The cells are scraped into 1 ml of cold PBS, collected by centrifugation (10,000 x g for 10 seconds at room temperature), and lysed by vortexing in 1 ml of lysis buffer (1% Nonidet P-40, 20 mM HEPES, pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.5% deoxycholate, 0.1% SDS, 1 mM DTT, 10 μ g/ml AEBSF, 10 μ g/ml aprotinin, 2 μ g/ml leupeptin and 2 μ g/ml antipain). The lysate is then centrifuged at 15,000 x g for 10 minutes at 4°C and the supernatant saved.

For immunoprecipitation of K4B-Ras, samples of lysate supernatant containing equal amounts of protein are utilized. Protein concentration is determined by the Bradford method utilizing bovine serum albumin as a standard. The appropriate volume of lysate is brought to 1 ml with lysis buffer lacking DTT and 8 μ g of

the pan Ras monoclonal antibody, Y13-259, added. The protein/antibody mixture is incubated on ice at 4°C for 24 hours. The immune complex is collected on pansorbin (Calbiochem) coated with rabbit antiserum to rat IgG (Cappel) by tumbling at 4°C for 45 minutes. The pellet is washed 3 times with 1 ml of lysis buffer lacking DTT and
5 protease inhibitors and resuspended in 100 µl elution buffer (10 mM Tris pH 7.4, 1% SDS). The Ras is eluted from the beads by heating at 95°C for 5 minutes, after which the beads are pelleted by brief centrifugation (15,000 x g for 30 seconds at room temperature).

The supernatant is added to 1 ml of Dilution Buffer 0.1% Triton X-
10 100, 5 mM EDTA, 50 mM NaCl, 10 mM Tris pH 7.4) with 2 µg Kirsten-ras specific monoclonal antibody, c-K-ras Ab-1 (Calbiochem). The second protein/antibody mixture is incubated on ice at 4°C for 1-2 hours. The immune complex is collected on pansorbin (Calbiochem) coated with rabbit antiserum to rat IgG (Cappel) by tumbling at 4°C for 45 minutes. The pellet is washed 3 times with 1 ml of lysis buffer
15 lacking DTT and protease inhibitors and resuspended in Laemmli sample buffer. The Ras is eluted from the beads by heating at 95°C for 5 minutes, after which the beads are pelleted by brief centrifugation. The supernatant is subjected to SDS-PAGE on a 12% acrylamide gel (bis-acrylamide:acrylamide, 1:100), and the Ras visualized by
20 fluorography.

hDJ processing inhibition assay

PSN-1 cells are seeded in 24-well assay plates. For each compound to be tested, the cells are treated with a minimum of seven concentrations in half-log steps. The final solvent (DMSO) concentration is 0.1%. A vehicle-only control is
25 included on each assay plate. The cells are treated for 24 hours at 37°C / 5% CO₂.

The growth media is then aspirated and the samples are washed with PBS. The cells are lysed with SDS-PAGE sample buffer containing 5% 2-mercaptoethanol and heated to 95°C for 5 minutes. After cooling on ice for 10 minutes, a mixture of nucleases is added to reduce viscosity of the samples.

30 The plates are incubated on ice for another 10 minutes. The samples are loaded onto pre-cast 8% acrylamide gels and electrophoresed at 15 mA/gel for 3-4 hours. The samples are then transferred from the gels to PVDF membranes by Western blotting.

The membranes are blocked for at least 1 hour in buffer containing 2%
35 nonfat dry milk. The membranes are then treated with a monoclonal antibody to hDJ-

2 (Neomarkers Cat. # MS-225), washed, and treated with an alkaline phosphatase-conjugated secondary antibody. The membranes are then treated with a fluorescent detection reagent and scanned on a phosphorimager.

5 For each sample, the percent of total signal corresponding to the unprenylated species of hDJ (the slower-migrating species) is calculated by densitometry. Dose-response curves and EC₅₀ values are generated using 4-parameter curve fits in SigmaPlot software.

EXAMPLE 73

10

Rap1 Processing Inhibition Assay

Protocol A:

15 Cells are labeled, incubated and lysed as described in Example 72. For immunoprecipitation of Rap1, samples of lysate supernatant containing equal amounts of protein are utilized. Protein concentration is determined by the Bradford method utilizing bovine serum albumin as a standard. The appropriate volume of lysate is brought to 1 ml with lysis buffer lacking DTT and 2 µg of the Rap1 antibody, Rap1/Krev1 (121) (Santa Cruz Biotech), is added. The protein/antibody mixture is incubated on ice at 4°C for 1 hour. The immune complex is collected on pansorbin (Calbiochem) by tumbling at 4°C for 45 minutes. The pellet is washed 3 times with 1 ml of lysis buffer lacking DTT and protease inhibitors and resuspended in 100 µl elution buffer (10 mM Tris pH 7.4, 1% SDS). The Rap1 is eluted from the beads by heating at 95°C for 5 minutes, after which the beads are pelleted by brief centrifugation (15,000 x g for 30 seconds at room temperature).

25 The supernatant is added to 1 ml of Dilution Buffer (0.1% Triton X-100, 5 mM EDTA, 50 mM NaCl, 10 mM Tris pH 7.4) with 2 µg Rap1 antibody, Rap1/Krev1 (121) (Santa Cruz Biotech). The second protein/antibody mixture is incubated on ice at 4°C for 1-2 hours. The immune complex is collected on pansorbin (Calbiochem) by tumbling at 4°C for 45 minutes. The pellet is washed 3 times with 1 ml of lysis buffer lacking DTT and protease inhibitors and resuspended in Laemmli sample buffer. The Rap1 is eluted from the beads by heating at 95°C for 5 minutes, after which the beads are pelleted by brief centrifugation. The supernatant is subjected to SDS-PAGE on a 12% acrylamide gel (bis-acrylamide:acrylamide, 1:100), and the Rap1 visualized by fluorography.

30

35

Protocol B:

PSN-1 cells are passaged every 3-4 days in 10cm plates, splitting near-confluent plates 1:20 and 1:40. The day before the assay is set up, 5×10^6 cells are plated on 15cm plates to ensure the same stage of confluency in each assay. The media for these cells is RPM1 1640 (Gibco), with 15% fetal bovine serum and 1x Pen/Strep antibiotic mix. The day of the assay, cells are collected from the 15cm plates by trypsinization and diluted to 400,000 cells/ml in media. 0.5ml of these diluted cells are added to each well of 24-well plates, for a final cell number of 200,000 per well. The cells are then grown at 37°C overnight.

The compounds to be assayed are diluted in DMSO in 1/2-log dilutions. The range of final concentrations to be assayed is generally 0.1-100 μ M. Four concentrations per compound is typical. The compounds are diluted so that each concentration is 1000x of the final concentration (i.e., for a 10 μ M data point, a 10 mM stock of the compound is needed).

2 μ L of each 1000x compound stock is diluted into 1 ml media to produce a 2X stock of compound. A vehicle control solution (2 μ L DMSO to 1ml media), is utilized. 0.5 ml of the 2X stocks of compound are added to the cells.

After 24 hours, the media is aspirated from the assay plates. Each well is rinsed with 1ml PBS, and the PBS is aspirated. 180 μ L SDS-PAGE sample buffer (Novex) containing 5% 2-mercapto-ethanol is added to each well. The plates are heated to 100°C for 5 minutes using a heat block containing an adapter for assay plates. The plates are placed on ice. After 10 minutes, 20 μ L of an RNase/DNase mix is added per well. This mix is 1mg/ml DNaseI (Worthington Enzymes), 0.25 mg/ml Rnase A (Worthington Enzymes), 0.5 M Tris-HCl pH 8.0 and 50 mM MgCl₂. The plate is left on ice for 10 minutes. Samples are then either loaded on the gel, or stored at -70°C until use.

Each assay plate (usually 3 compounds, each in 4-point titrations, plus controls) requires one 15-well 14% Novex gel. 25 μ l of each sample is loaded onto the gel. The gel is run at 15 mA for about 3.5 hours. It is important to run the gel far enough so that there will be adequate separation between 21 kd (Rap1) and 29 kd (Rab6).

The gels are then transferred to Novex pre-cut PVDF membranes for 1.5 hours at 30V (constant voltage). Immediately after transferring, the membranes are blocked overnight in 20ml Western blocking buffer (2% nonfat dry milk in

Western wash buffer (PBS + 0.1% Tween-20). If blocked over the weekend, 0.02% sodium azide is added. The membranes are blocked at 4°C with slow rocking.

The blocking solution is discarded and 20ml fresh blocking solution containing the anti Rap1a antibody (Santa Cruz Biochemical SC1482) at 1:1000
5 (diluted in Western blocking buffer) and the anti Rab6 antibody (Santa Cruz Biochemical SC310) at 1:5000 (diluted in Western blocking buffer) are added. The membranes are incubated at room temperature for 1 hour with mild rocking. The blocking solution is then discarded and the membrane is washed 3 times with Western wash buffer for 15 minutes per wash. 20ml blocking solution containing 1:1000
10 (diluted in Western blocking buffer) each of two alkaline phosphatase conjugated antibodies (Alkaline phosphatase conjugated Anti-goat IgG and Alkaline phosphatase conjugated anti-rabbit IgG [Santa Cruz Biochemical]) is then added. The membrane is incubated for one hour and washed 3x as above.

About 2 ml per gel of the Amersham ECF detection reagent is placed
15 on an overhead transparency (ECF) and the PVDF membranes are placed face down onto the detection reagent. This is incubated for one minute, then the membrane is placed onto a fresh transparency sheet.

The developed transparency sheet is scanned on a phosphorimager and the Rap1a Minimum Inhibitory Concentration is determined from the lowest
20 concentration of compound that produces a detectable Rap1a Western signal. The Rap1a antibody used recognizes only unprenylated/unprocessed Rap1a, so that the presence of a detectable Rap1a Western signal is indicative of inhibition of Rap1a prenylation.

25 Protocol C:

This protocol allows the determination of an EC₅₀ for inhibition of processing of Rap1a. The assay is run as described in Protocol B with the following modifications. 20 µl of sample is run on pre-cast 10-20% gradient acrylamide mini gels (Novex Inc.) at 15 mA/gel for 2.5-3 hours. Prenylated and unprenylated forms
30 of Rap1a are detected by blotting with a polyclonal antibody (Rap1/Krev-1 Ab#121; Santa Cruz Research Products #sc-65), followed by an alkaline phosphatase-conjugated anti-rabbit IgG antibody. The percentage of unprenylated Rap1a relative to the total amount of Rap1a is determined by peak integration using Imagequant™ software (Molecular Dynamics). Unprenylated Rap1a is distinguished from prenyl-

ated protein by virtue of the greater apparent molecular weight of the prenylated protein. Dose-response curves and EC₅₀ values are generated using 4-parameter curve fits in SigmaPlot software.

5

EXAMPLE 75

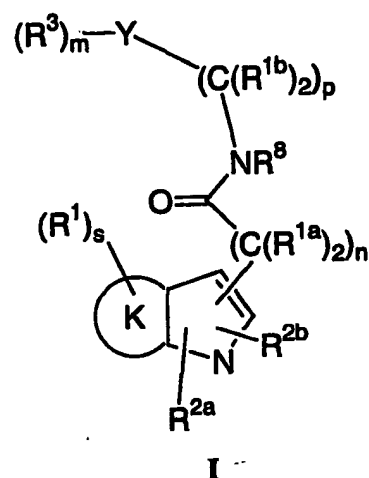
In vivo tumor growth inhibition assay (nude mouse)

In vivo efficacy as an inhibitor of the growth of cancer cells may be confirmed by several protocols well known in the art. Examples of such *in vivo* efficacy studies are described by N. E. Kohl et al. (Nature Medicine, 1:792-797 (1995)) and N. E. Kohl et al. (Proc. Nat. Acad. Sci. U.S.A., 91:9141-9145 (1994)).

Rodent fibroblasts transformed with oncogenically mutated human Ha-ras or Ki-ras (10⁶ cells/animal in 1 ml of DMEM salts) are injected subcutaneously into the left flank of 8-12 week old female nude mice (Harlan) on day 0. The mice in each oncogene group are randomly assigned to a vehicle or compound treatment group. Animals are dosed subcutaneously starting on day 1 and daily for the duration of the experiment. Alternatively, the farnesyl-protein transferase inhibitor may be administered by a continuous infusion pump. Compound or vehicle is delivered in a total volume of 0.1 ml. Tumors are excised and weighed when all of the vehicle-treated animals exhibited lesions of 0.5-1.0 cm in diameter, typically 11-15 days after the cells were injected. The average weight of the tumors in each treatment group for each cell line is calculated.

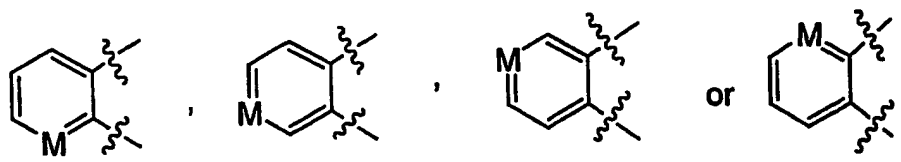
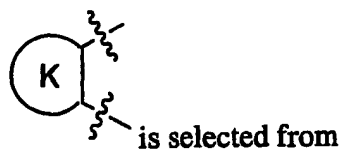
WHAT IS CLAIMED IS:

1. A compound of the formula I:



5

wherein



10

M is C or N;

R¹ is independently selected from

15

- a) H,
- b) unsubstituted or substituted C₁-C₆ alkyl,
- c) unsubstituted or substituted C₁-C₆ alkoxy,
- d) unsubstituted or substituted aryl,
- e) unsubstituted or substituted C₂-C₈ alkenyl,

- 5
- f) unsubstituted or substituted C₂-C₈ alkynyl,
 - g) unsubstituted or substituted perfluoroalkyl,
 - h) halo,
 - i) OR¹⁰,
 - j) R¹¹S(O)_m,
 - k) R¹⁰C(O)NR¹⁰-,
 - l) -C(O)N(R¹⁰)₂,
 - m) CN,
 - n) NO₂,
 - 10 o) R¹⁰C(O)-,
 - p) R¹⁰OC(O)-,
 - q) N₃,
 - r) -N(R¹⁰)₂, or
 - s) R¹¹OC(O)NR¹⁰-;

15

R^{1a} and R^{1b} are independently selected from:

- 20
- a) H,
 - b) unsubstituted or substituted C₁-C₆ alkyl,
 - c) unsubstituted or substituted C₁-C₆ alkoxy,
 - d) unsubstituted or substituted aryl,
 - e) unsubstituted or substituted heterocycle,
 - f) unsubstituted or substituted aralkyl, or
 - g) -(CH₂)_n heterocycle;

25 R^{2a} and R^{2b} are independently selected from:

- 30
- a) H,
 - b) unsubstituted or substituted C₁-C₆ alkyl,
 - c) unsubstituted or substituted aryl,
 - d) oxo,
 - e) unsubstituted or substituted heterocycle, or
 - f) unsubstituted or substituted C₁-C₆ alkoxy;

wherein the substituted group is substituted with from one to three substituents selected from:

- 1) unsubstituted or substituted C₁-C₆ alkyl,
- 2) unsubstituted or substituted C₁-C₆ alkoxy,
- 3) halo,
- 4) unsubstituted or substituted aryl,
- 5) unsubstituted or substituted heterocycle,
- 6) unsubstituted or substituted C₂-C₈ alkenyl,
- 7) unsubstituted or substituted C₂-C₈ alkynyl,
- 8) unsubstituted or substituted perfluoroalkyl,
- 9) -OR¹⁰,
- 10) R¹¹S(O)_m-,
- 11) R¹⁰C(O)NR¹⁰-,
- 12) -C(O)N(R¹⁰)₂,
- 13) CN,
- 14) NO₂,
- 15) -N(R¹⁰)₂,
- 16) R¹⁰C(O)-,
- 17) R¹⁰OC(O)-,
- 18) N₃, or
- 19) R¹¹OC(O)NR¹⁰-;

20

R^{2a} and R^{2b} may optionally be joined in a ring;

R³ is independently selected from:

- a) H,
- 25 b) unsubstituted or substituted C₁-C₆ alkyl,
- c) unsubstituted or substituted C₂-C₈ alkenyl,
- d) unsubstituted or substituted C₂-C₈ alkynyl,
- e) unsubstituted or substituted perfluoroalkyl,
- f) halo,
- 30 g) -OR¹⁰,
- h) R¹¹S(O)_m-,
- i) R¹⁰C(O)NR¹⁰-,
- j) -C(O)NR¹⁰,
- k) CN,

- 5
- l) NO₂,
 - m) R¹⁰C(O)-,
 - n) R¹⁰OC(O)-,
 - o) N₃,
 - p) -N(R¹⁰)₂, or
 - q) R¹¹OC(O)NR¹⁰-;

R⁸ is selected from:

- 10
- a) unsubstituted or substituted C₁-C₆ alkyl,
 - b) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
 - c) unsubstituted or substituted aryl,
 - d) unsubstituted or substituted C₂-C₈ alkenyl, or
 - e) unsubstituted or substituted C₂-C₈ alkynyl;

wherein the substituted group is selected from:

- 15
- 1) unsubstituted or substituted C₁-C₆ alkyl,
 - 2) unsubstituted or substituted C₂-C₈ alkenyl,
 - 3) unsubstituted or substituted C₂-C₈ alkynyl,
 - 4) unsubstituted or substituted C₁-C₆ alkoxy,
 - 5) CN,
 - 6) unsubstituted or substituted aryl, or
 - 7) unsubstituted or substituted heterocycle;
- 20

R¹⁰ is selected from:

- 25
- a) H,
 - b) unsubstituted or substituted C₁-C₆ alkyl,
 - c) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
 - d) unsubstituted or substituted aryl,
 - e) unsubstituted or substituted heterocycle,
 - f) unsubstituted or substituted aralkyl, or
 - 30 g) unsubstituted or substituted heterocyclalkyl;

R¹¹ is selected from

- a) unsubstituted or substituted C₁-C₆ alkyl,
- b) unsubstituted or substituted C₃-C₁₀ cycloalkyl,

- c) unsubstituted or substituted aryl,
- d) unsubstituted or substituted heterocycle,
- e) unsubstituted or substituted aralkyl, or
- f) unsubstituted or substituted heterocyclalkyl;

5

Y is selected from:

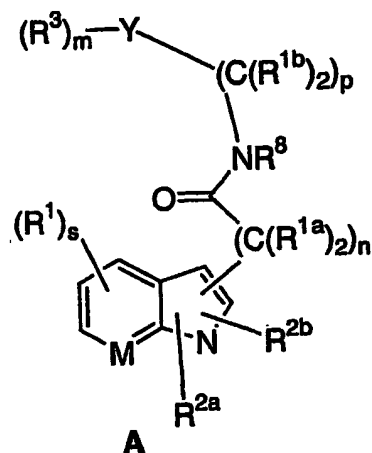
- a) heterocycle,
- b) C₃-C₁₀ cycloalkyl,
- c) C₂-C₈ alkenyl,
- 10 d) C₂-C₈ alkynyl,
- e) C₁-C₆ alkoxy,
- f) CN,
- g) C(O),
- h) C(O)OR¹⁰,
- 15 i) -OR¹⁰,
- j) -N(R¹⁰)₂, or
- k) C(=NH)NHR¹⁰;

- m is 0 to 4;
- 20 n is 0 to 4;
- p is 1 to 4;
- s is 0 to 4;

or a pharmaceutically acceptable salt or stereoisomer thereof.

25

2. The compound according to Claim 1 of formula A:



wherein

5 M is C or N;

R¹ is independently selected from

- a) H,
- b) unsubstituted or substituted C₁-C₆ alkyl,
- 10 c) unsubstituted or substituted C₁-C₆ alkoxy,
- d) unsubstituted or substituted aryl,
- e) unsubstituted or substituted C₂-C₈ alkenyl,
- f) unsubstituted or substituted C₂-C₈ alkynyl,
- g) unsubstituted or substituted perfluoroalkyl,
- 15 h) halo,
- i) OR¹⁰,
- j) R¹¹S(O)_m,
- k) R¹⁰C(O)NR¹⁰-,
- l) -C(O)N(R¹⁰)₂,
- 20 m) CN,
- n) NO₂,
- o) R¹⁰C(O)-,
- p) R¹⁰OC(O)-,
- q) N₃,
- 25 r) -N(R¹⁰)₂, or

s) $R^{11}OC(O)NR^{10}$;

R^{1a} is independently selected from:

- a) H,
- 5 b) unsubstituted or substituted C_1 - C_6 alkyl,
- c) unsubstituted or substituted C_1 - C_6 alkoxy,
- d) unsubstituted or substituted aryl, or
- e) unsubstituted or substituted heterocycle;

10 R^{1b} is independently selected from:

- a) H,
- b) unsubstituted or substituted C_1 - C_6 alkyl,
- c) unsubstituted or substituted C_1 - C_6 alkoxy,
- d) unsubstituted or substituted aryl,
- 15 e) unsubstituted or substituted heterocycle,
- f) unsubstituted or substituted aralkyl, or
- g) $-(CH_2)_n$ heterocycle;

R^{2a} and R^{2b} are independently selected from:

- 20 a) H,
- b) unsubstituted or substituted C_1 - C_6 alkyl,
- c) unsubstituted or substituted aryl,
- d) oxo,
- e) unsubstituted or substituted heterocycle, or
- 25 f) unsubstituted or substituted C_1 - C_6 alkoxy;

wherein the substituted group is substituted with from one to three substituents selected from:

- 1) unsubstituted or substituted C_1 - C_6 alkyl,
- 2) unsubstituted or substituted C_1 - C_6 alkoxy,
- 30 3) halo,
- 4) unsubstituted or substituted aryl,
- 5) unsubstituted or substituted heterocycle,
- 6) unsubstituted or substituted C_2 - C_8 alkenyl,
- 7) unsubstituted or substituted C_2 - C_8 alkynyl,

- 5
- 8) unsubstituted or substituted perfluoroalkyl,
 - 9) -OR¹⁰,
 - 10) R¹¹S(O)_m-,
 - 11) R¹⁰C(O)NR¹⁰-,
 - 12) -C(O)N(R¹⁰)₂,
 - 13) CN,
 - 14) NO₂,
 - 15) -N(R¹⁰)₂,
 - 16) R¹⁰C(O)-,
 - 10 17) R¹⁰OC(O)-,
 - 18) N₃, or
 - 19) R¹¹OC(O)NR¹⁰-;

R^{2a} and R^{2b} may optionally be joined in a ring;

15

R³ is independently selected from:

- a) H,
- b) unsubstituted or substituted C₁-C₆ alkyl,
- c) unsubstituted or substituted C₂-C₈ alkenyl,
- 20 d) unsubstituted or substituted C₂-C₈ alkynyl,
- e) unsubstituted or substituted perfluoroalkyl,
- f) halo,
- g) -OR¹⁰,
- h) R¹¹S(O)_m-,
- 25 i) R¹⁰C(O)NR¹⁰-,
- j) -C(O)NR¹⁰,
- k) CN,
- l) NO₂,
- m) R¹⁰C(O)-,
- 30 n) R¹⁰OC(O)-,
- o) N₃,
- p) -N(R¹⁰)₂, or
- q) R¹¹OC(O)NR¹⁰-;

R⁸ is selected from:

- a) unsubstituted or substituted C₁-C₆ alkyl,
- b) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
- c) unsubstituted or substituted aryl,
- 5 d) unsubstituted or substituted C₂-C₈ alkenyl, or
- e) unsubstituted or substituted C₂-C₈ alkynyl;

wherein the substituted group is selected from:

- 1) unsubstituted or substituted C₁-C₆ alkyl,
- 2) unsubstituted or substituted C₂-C₈ alkenyl,
- 10 3) unsubstituted or substituted C₂-C₈ alkynyl,
- 4) unsubstituted or substituted C₁-C₆ alkoxy,
- 5) CN,
- 6) unsubstituted or substituted aryl, or
- 7) unsubstituted or substituted heterocycle;

15

R¹⁰ is selected from:

- a) H,
- b) unsubstituted or substituted C₁-C₆ alkyl,
- c) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
- 20 d) unsubstituted or substituted aryl,
- e) unsubstituted or substituted heterocycle,
- f) unsubstituted or substituted aralkyl, or
- g) unsubstituted or substituted heterocyclalkyl;

25 R¹¹ is selected from

- a) unsubstituted or substituted C₁-C₆ alkyl,
- b) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
- c) unsubstituted or substituted aryl,
- d) unsubstituted or substituted heterocycle,
- 30 e) unsubstituted or substituted aralkyl, or
- f) unsubstituted or substituted heterocyclalkyl;

Y is selected from:

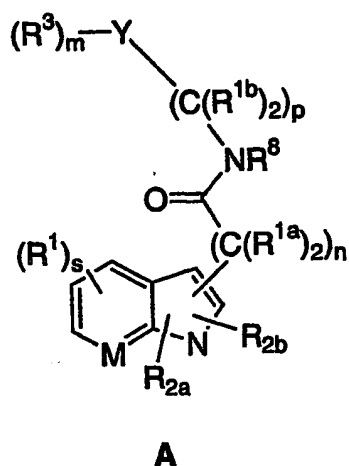
- 5 a) heterocycle,
 b) C₃-C₁₀ cycloalkyl,
 c) C₂-C₈ alkenyl,
 d) C₂-C₈ alkynyl,
 e) C₁-C₆ alkoxy,
 f) CN,
 g) C(O),
 h) C(O)OR¹⁰,
 10 i) -OR¹⁰,
 j) -N(R¹⁰)₂, or
 k) C(=NH)NHR¹⁰;

- m is 0 to 4;
 15 n is 0 or 1;
 p is 1 to 4;
 s is 0 to 4;

or a pharmaceutically acceptable salt or stereoisomer thereof.

20

3. The compound according to Claim 1 of formula A:



25 wherein

M is C or N;

R¹ is independently selected from

- 5 a) H,
 b) unsubstituted or substituted C₁-C₆ alkyl,
 c) unsubstituted or substituted C₁-C₆ alkoxy,
 d) unsubstituted or substituted aryl,
 e) unsubstituted or substituted perfluoroalkyl,
10 f) halo,
 g) OR¹⁰,
 h) R¹⁰C(O)NR¹⁰-,
 i) -C(O)N(R¹⁰)₂,
 j) CN,
15 k) R¹⁰C(O)-,
 l) R¹⁰OC(O)-,
 m) -N(R¹⁰)₂, or
 n) R¹¹OC(O)NR¹⁰-;

20 R^{1a} is independently selected from:

- a) H,
 b) unsubstituted or substituted C₁-C₆ alkyl, or
 c) unsubstituted or substituted C₁-C₆ alkoxy;

25 R^{1b} is independently selected from:

- a) H,
 b) unsubstituted or substituted C₁-C₆ alkyl,
 c) unsubstituted or substituted C₁-C₆ alkoxy,
 d) unsubstituted or substituted aryl,
30 e) unsubstituted or substituted heterocycle,
 f) unsubstituted or substituted aralkyl, or
 g) -(CH₂)_n heterocycle;

R^{2a} and R^{2b} are independently selected from:

- 5
- a) H,
 - b) unsubstituted or substituted C₁-C₆ alkyl,
 - c) unsubstituted or substituted aryl,
 - d) oxo,
 - e) unsubstituted or substituted heterocycle, or
 - f) unsubstituted or substituted C₁-C₆ alkoxy;

wherein the substituted group is substituted with from one to three substituents selected from:

- 10
- 1) unsubstituted or substituted C₁-C₆ alkyl,
 - 2) unsubstituted or substituted C₁-C₆ alkoxy,
 - 3) halo,
 - 4) unsubstituted or substituted aryl,
 - 5) unsubstituted or substituted heterocycle,
 - 6) unsubstituted or substituted perfluoroalkyl,
 - 15 7) -OR¹⁰,
 - 8) R¹⁰C(O)NR¹⁰-,
 - 9) -C(O)N(R¹⁰)₂,
 - 10) CN,
 - 11) NO₂, or
 - 20 12) -N(R¹⁰)₂;

R^{2a} and R^{2b} may optionally be joined in a ring;

R³ is independently selected from:

- 25
- a) H,
 - b) unsubstituted or substituted C₁-C₆ alkyl,
 - c) halo,
 - d) -OR¹⁰,
 - e) R¹¹S(O)_m-,
 - 30 f) R¹⁰C(O)NR¹⁰-,
 - g) -C(O)NR¹⁰,
 - h) R¹⁰C(O)-,
 - i) -N(R¹⁰)₂, or
 - j) R¹¹OC(O)NR¹⁰-;
 - 35

R8 is selected from:

- a) unsubstituted or substituted C₁-C₆ alkyl,
- b) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
- c) unsubstituted or substituted aryl,
- 5 d) unsubstituted or substituted C₂-C₈ alkenyl, or
- e) unsubstituted or substituted C₂-C₈ alkynyl;

wherein the substituted group is selected from:

- 1) unsubstituted or substituted C₁-C₆ alkyl,
- 2) unsubstituted or substituted C₂-C₈ alkenyl,
- 10 3) unsubstituted or substituted C₂-C₈ alkynyl,
- 4) unsubstituted or substituted C₁-C₆ alkoxy,
- 5) CN,
- 6) unsubstituted or substituted aryl, or
- 7) unsubstituted or substituted heterocycle;

15

R10 is selected from:

- a) H,
- b) unsubstituted or substituted C₁-C₆ alkyl,
- c) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
- 20 d) unsubstituted or substituted aryl,
- e) unsubstituted or substituted heterocycle,
- f) unsubstituted or substituted aralkyl, or
- g) unsubstituted or substituted heterocyclalkyl;

25 R11 is selected from

- a) unsubstituted or substituted C₁-C₆ alkyl,
- b) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
- c) unsubstituted or substituted aryl,
- d) unsubstituted or substituted heterocycle,
- 30 e) unsubstituted or substituted aralkyl, or
- f) unsubstituted or substituted heterocyclalkyl;

Y is selected from:

- a) heterocycle,
 b) C₃-C₁₀ cycloalkyl,
 c) C₂-C₈ alkenyl,
 d) C₂-C₈ alkynyl,
 5 e) C₁-C₆ alkoxy,
 f) CN,
 g) C(O),
 h) C(O)OR¹⁰,
 i) -OR¹⁰,
 10 j) -N(R¹⁰)₂, or
 k) C(=NH)NHR¹⁰;

m is 0 to 4;

n is 0 or 1;

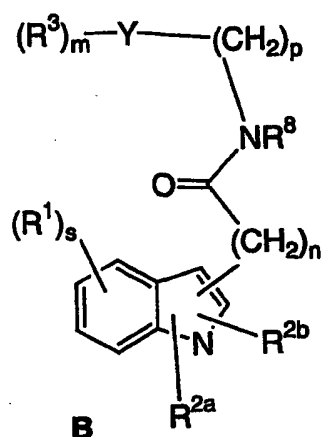
15 p is 1 to 4;

s is 0 to 4;

or a pharmaceutically acceptable salt or stereoisomer thereof.

4. The compound according to Claim 1 of formula B:

20



wherein

25 R¹ is independently selected from

- 5
- a) H,
 - b) unsubstituted or substituted C₁-C₆ alkyl,
 - c) unsubstituted or substituted C₁-C₆ alkoxy,
 - d) unsubstituted or substituted aryl,
 - e) unsubstituted or substituted perfluoroalkyl,
 - f) halo,
 - g) OR¹⁰,
 - h) R¹⁰C(O)NR¹⁰-,
 - i) -C(O)N(R¹⁰)₂,
 - 10 j) CN,
 - k) R¹⁰C(O)-, or
 - l) -N(R¹⁰)₂;

R^{2a} and R^{2b} are independently selected from:

- 15
- a) H,
 - b) unsubstituted or substituted C₁-C₆ alkyl,
 - c) unsubstituted or substituted aryl,
 - d) oxo,
 - e) unsubstituted or substituted heterocycle, or
 - 20 f) unsubstituted or substituted C₁-C₆ alkoxy;

wherein the substituted group is substituted with from one to three substituents selected from:

- 25
- 1) unsubstituted or substituted C₁-C₆ alkyl,
 - 2) unsubstituted or substituted C₁-C₆ alkoxy,
 - 3) halo,
 - 4) unsubstituted or substituted aryl,
 - 5) unsubstituted or substituted heterocycle,
 - 6) unsubstituted or substituted perfluoroalkyl,
 - 7) -OR¹⁰,
 - 30 8) R¹⁰C(O)NR¹⁰-,
 - 9) -C(O)N(R¹⁰)₂, or
 - 10) -N(R¹⁰)₂;

R^{2a} and R^{2b} may optionally be joined in a ring;

35

R³ is independently selected from:

- a) H,
- b) unsubstituted or substituted C₁-C₆ alkyl,
- c) halo,
- 5 d) -OR¹⁰,
- e) R¹⁰C(O)NR¹⁰-,
- f) -C(O)NR¹⁰,
- g) R¹⁰C(O)-, or
- h) -N(R¹⁰)₂;

10

R⁸ is selected from:

- a) unsubstituted or substituted C₁-C₆ alkyl,
- b) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
- c) unsubstituted or substituted aryl,
- 15 d) unsubstituted or substituted C₂-C₈ alkenyl, or
- e) unsubstituted or substituted C₂-C₈ alkynyl;

wherein the substituted group is selected from:

- 1) unsubstituted or substituted C₁-C₆ alkyl,
- 2) unsubstituted or substituted C₂-C₈ alkenyl,
- 20 3) unsubstituted or substituted C₂-C₈ alkynyl,
- 4) unsubstituted or substituted C₁-C₆ alkoxy,
- 5) CN,
- 6) unsubstituted or substituted aryl, or
- 7) unsubstituted or substituted heterocycle;

25

R¹⁰ is selected from:

- a) H,
- b) unsubstituted or substituted C₁-C₆ alkyl,
- c) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
- 30 d) unsubstituted or substituted aryl,
- e) unsubstituted or substituted heterocycle,
- f) unsubstituted or substituted aralkyl, or
- g) unsubstituted or substituted heterocyclalkyl;

R¹¹ is selected from

- a) unsubstituted or substituted C₁-C₆ alkyl,
- b) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
- c) unsubstituted or substituted aryl,
- 5 d) unsubstituted or substituted heterocycle,
- e) unsubstituted or substituted aralkyl, or
- f) unsubstituted or substituted heterocyclalkyl;

Y is selected from:

- 10 a) heterocycle, which is selected from pyridinyl, furanyl, pyrazolyl, pyrimidinyl, pyrazinyl, imidazolyl, oxazolyl, thiazolyl, triazolyl, tetrazolyl or thiofuranyl;
- b) C₃-C₁₀ cycloalkyl,
- c) C₂-C₈ alkenyl,
- 15 d) C₂-C₈ alkynyl,
- e) C₁-C₆ alkoxy,
- f) CN,
- g) C(O),
- h) C(O)OR¹⁰,
- 20 i) -OR¹⁰,
- j) -N(R¹⁰)₂, or
- k) C(=NH)NHR¹⁰;

m is 0 to 4;

25 n is 0 or 1;

p is 1 to 4;

s is 0 to 4;

or a pharmaceutically acceptable salt or stereoisomer thereof.

30

5. A compound which is selected from:

N-isopropyl-2-(1-methyl-2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide;

N-isopropyl-2-(1-methyl-2-o-tolyl-1H-indol-3-yl)-N-pyridin-4-ylmethyl-acetamide;

- N-isopropyl-2-(7-methyl-2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide;
 2-[1-(2-bromobenzyl)-1H-indol-3-yl]-N-isopropyl-N-(pyridin-4-ylmethyl)acetamide;
 N-isopropyl-2-(2-phenyl-1H-indol-1-yl)-N-(pyridin-4-ylmethyl)acetamide;
 N-isopropyl-2-(2-phenyl-1H-pyrrolo[2,3-b]pyridin-3-yl)-N-pyridin-4-ylmethyl-
 5 acetamide;
 N-benzyl-N-isopropyl-2-(2-phenyl-1H-indol-3-yl)acetamide;
 N-ethyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide;
 2-[1-(4-bromobenzyl)-1H-indol-3-yl]-N-isopropyl-N-(pyridin-4-ylmethyl)acetamide;
 2-[1-(3-bromobenzyl)-1H-indol-3-yl]-N-isopropyl-N-(pyridin-4-ylmethyl)acetamide;
 10 2-(2-phenyl-1H-indol-3-yl)-N,N-bis(pyridin-3-ylmethyl)acetamide;
 2-(1-benzyl-1H-indol-3-yl)-N-isopropyl-N-(pyridin-4-ylmethyl)acetamide;
 2-[2-(4-bromophenyl)-1H-indol-3-yl]-N-isopropyl-N-(pyridin-4-ylmethyl)acetamide;
 2-(1-ethyl-2-phenyl-1H-indol-3-yl)-N-isopropyl-N-(pyridin-4-ylmethyl)acetamide;
 N-isopropyl-2-(2-phenyl-1-propyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide;
 15 2-(1-benzyl-2-oxo-2,3-dihydro-1H-indol-3-yl)-N-isopropyl-N-(pyridin-4-ylmethyl)
 acetamide;
 1-benzyl-4-[[[(1-benzyl-2-oxo-2,3-dihydro-1H-indol-3-yl)acetyl] (isopropyl)amino]
 methyl}pyridinium;
 N-cyclobutyl-2-(2-phenyl-1H-indol-3-yl)-N-pyridin-3-ylmethyl-acetamide;
 20 N-cyclopropyl-2-(2-phenyl-1H-indol-3-yl)-N-pyridin-3-ylmethyl-acetamide;
 2-[2-(2-chloro-phenyl)-1H-indol-3-yl]-N-isopropyl-N-pyridin-4-ylmethyl-acetamide;
 N,N-diallyl-2-(2-phenyl-1H-indol-3-yl)-acetamide;
 N-isopropyl-2-(2-phenyl-1H-indol-3-yl)-N-pyridin-3-ylmethyl-acetamide;
 (S)-N-sec-butyl-2-(2-phenyl-1H-indol-3-yl)-N-pyridin-4-ylmethyl-acetamide;
 25 N-furan-3-ylmethyl-N-isopropyl-2-(2-phenyl-1H-indol-3-yl)-acetamide;
 N-(2-cyano-ethyl)-N-cyclopropyl-2-(2-phenyl-1H-indol-3-yl)-acetamide;
 N-cyclobutyl-2-(2-phenyl-1H-indol-3-yl)-N-pyridin-4-ylmethyl-acetamide;
 N-cyclopropyl-2-(2-phenyl-1H-indol-3-yl)-N-pyridin-4-ylmethyl-acetamide;
 2-[2-(3-chloro-phenyl)-1H-indol-3-yl]-N-isopropyl-N-pyridin-4-ylmethyl-acetamide;
 30 N-(2-cyano-ethyl)-N-cyclopropyl-2-(1-methyl-2-phenyl-1H-indol-3-yl)-acetamide;
 N-isopropyl-2-(2-phenyl-1H-indol-3-yl)-N-pyrimidin-5-ylmethyl-acetamide;
 N-cyclopropyl-N-(2-methyl-2H-pyrazol-3-ylmethyl)-2-(2-phenyl-1H-indol-3-yl)-
 acetamide;
 (+)-N-isopropyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)propanamide;
 35 N-(2-furylmethyl)-N-isopropyl-2-(2-phenyl-1H-indol-3-yl)acetamide;

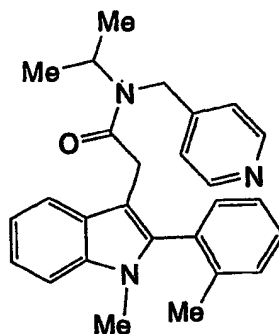
- N-isopropyl-N-[(1-methyl-1H-pyrazol-5-yl)methyl]-2-(2-phenyl-1H-indol-3-yl)acetamide;
- N-isobutyl-N-methyl-2-(2-phenyl-1H-indol-3-yl)acetamide;
- N-cyclopropyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyrazin-2-ylmethyl)acetamide;
- 5 N-cyclopropyl-N-(3-furylmethyl)-2-(2-phenyl-1H-indol-3-yl)acetamide;
- N-(2-cyanoethyl)-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-3-ylmethyl)acetamide;
- N-isopropyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyrazin-2-ylmethyl)acetamide;
- N-isopropyl-N-[(1-methyl-1H-pyrazol-4-yl)methyl]-2-(2-phenyl-1H-indol-3-yl)acetamide;
- 10 N-isopropyl-N-[(1-methyl-1H-pyrazol-3-yl)methyl]-2-(2-phenyl-1H-indol-3-yl)acetamide;
- N-isopropyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-2-ylmethyl)acetamide;
- N-(2-cyanoethyl)-N-cyclopropyl-2-[2-(2-methylphenyl)-1H-indol-3-yl]acetamide;
- N-isopentyl-N-methyl-2-(2-phenyl-1H-indol-3-yl)acetamide;
- 15 N-(2-cyanoethyl)-N-isopropyl-2-(2-phenyl-1H-indol-3-yl)acetamide;
- N-cyclopropyl-N-[(1-methyl-1H-pyrazol-4-yl)methyl]-2-(2-phenyl-1H-indol-3-yl)acetamide;
- N-cyclopropyl-N-[(1-methyl-1H-pyrazol-3-yl)methyl]-2-(2-phenyl-1H-indol-3-yl)acetamide;
- 20 N-cyclobutyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-2-ylmethyl)acetamide;
- N-cyclopropyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-2-ylmethyl)acetamide;
- N-cyclopropyl-N-(2-furylmethyl)-2-(2-phenyl-1H-indol-3-yl)acetamide;
- N-isopropyl-2-[2-(2-methoxyphenyl)-1H-indol-3-yl]-N-(pyridin-4-ylmethyl)acetamide;
- 25 N-isobutyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-2-ylmethyl)acetamide;
- 2-[2-(2-chlorophenyl)-1H-indol-3-yl]-N-(2-cyanoethyl)-N-cyclopropylacetamide;
- N-(tert-butyl)-N-(2-cyanoethyl)-2-(2-phenyl-1H-indol-3-yl)acetamide;
- N-isobutyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-3-ylmethyl)acetamide;
- methyl N-cyclopropyl-N-[(2-phenyl-1H-indol-3-yl)acetyl]-beta-alaninate;
- 30 N-isopropyl-2-(6-methyl-2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide;
- N-isopropyl-2-(4-methyl-2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide;
- N-isobutyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide;
- N-cyclopentyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide;
- N-[(1R)-1-methylpropyl]-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide;
- 35 acetamide;

- N,N-bis(2-methoxyethyl)-2-(2-phenyl-1H-indol-3-yl)acetamide;
 N-isopropyl-2-(5-methyl-2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide;
 N-(2-cyanoethyl)-N-cyclopropyl-2-[2-(2-methoxyphenyl)-1H-indol-3-yl]acetamide;
 N-cyclopentyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-2-ylmethyl)acetamide;
 5 N-cyclopentyl-2-(2-phenyl-1H-indol-3-yl)-N-(pyridin-3-ylmethyl)acetamide;
 N-isopropyl-N-phenyl-2-(2-phenyl-1H-indol-3-yl)acetamide;
 (2-phenyl-1H-indol-3-yl)acetyl N-[3-(dimethylamino)propyl]-N'-ethylimidocarbamate;
 N-cyclopropyl-N-[(2-phenyl-1H-indol-3-yl)acetyl]-beta-alanine;
 10 N-Isopropyl-2-(2-pyridin-3-yl-1H-indol-3-yl)-N-pyridin-4-ylmethyl-acetamide;
 N-Isopropyl-2-(2-pyridin-4-yl-1H-indol-3-yl)-N-pyridin-3-ylmethyl-acetamide;
 N-Benzyl-N-isopropyl-2-(2-pyridin-4-yl-1H-indol-3-yl)-acetamide;

or a pharmaceutically acceptable salt or stereoisomer thereof.

15

6. The compound according to Claim 5 which is:

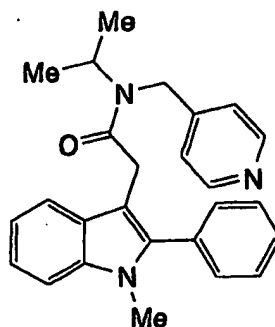


- 20 N-isopropyl-2-(1-methyl-2-o-tolyl-1H-indol-3-yl)-N-pyridin-4-ylmethyl-acetamide

or a pharmaceutically acceptable salt or stereoisomer thereof.

7. The compound according to Claim 5 which is:

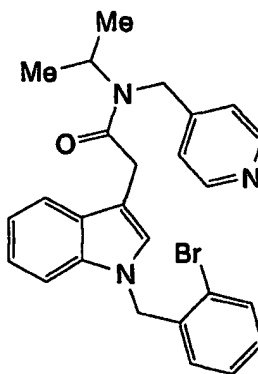
25



N-isopropyl-2-(1-methyl-2-phenyl-1H-indol-3-yl)-N-(pyridin-4-ylmethyl)acetamide

5 or a pharmaceutically acceptable salt or stereoisomer thereof.

8. The compound according to Claim 5 which is:



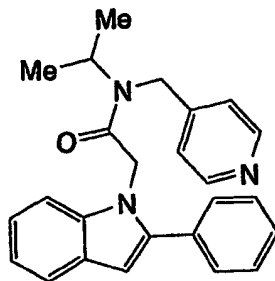
10

2-[1-(2-bromobenzyl)-1H-indol-3-yl]-N-isopropyl-N-(pyridin-4-ylmethyl)acetamide

or a pharmaceutically acceptable salt or stereoisomer thereof.

15

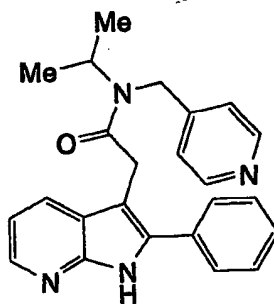
9. The compound according to Claim 5 which is:



N-isopropyl-2-(2-phenyl-1H-indol-1-yl)-N-(pyridin-4-ylmethyl)acetamide

5 or a pharmaceutically acceptable salt or stereoisomer thereof.

10. The compound according to Claim 5 which is:



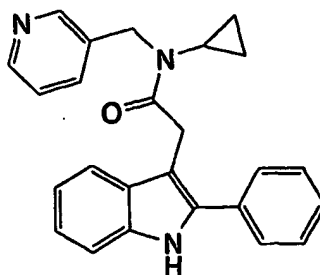
10

N-isopropyl-2-(2-phenyl-1H-pyrrolo[2,3-b]pyridin-3-yl)-N-(pyridin-4-ylmethyl)-
acetamide

or a pharmaceutically acceptable salt or stereoisomer thereof.

15

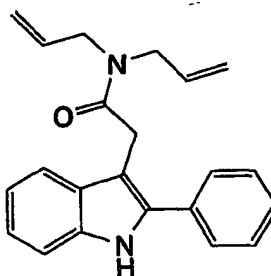
11. The compound according to Claim 5 which is:



N-cyclopropyl-2-(2-phenyl-1H-indol-3-yl)-N-pyridin-3-ylmethyl-acetamide

5 or a pharmaceutically acceptable salt or stereoisomer thereof.

12. The compound according to Claim 5 which is:



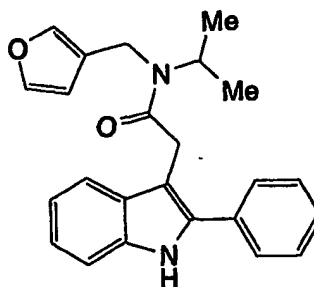
10

N,N-diallyl-2-(2-phenyl-1H-indol-3-yl)-acetamide

or a pharmaceutically acceptable salt or stereoisomer thereof.

15

13. The compound according to Claim 5 which is:

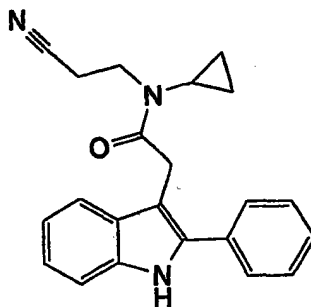


N-furan-3-ylmethyl-N-isopropyl-2-(2-phenyl-1H-indol-3-yl)-acetamide

or a pharmaceutically acceptable salt or stereoisomer thereof.

5

14. The compound according to Claim 5 which is:



10 N-(2-cyano-ethyl)-N-cyclopropyl-2-(2-phenyl-1H-indol-3-yl)-acetamide

or a pharmaceutically acceptable salt or stereoisomer thereof.

15 15. A pharmaceutical composition comprising a pharmaceutical carrier, and dispersed therein, a therapeutically effective amount of a compound of Claim 1.

16 16. A pharmaceutical composition comprising a pharmaceutical carrier, and dispersed therein, a therapeutically effective amount of a compound of Claim 3.

17. A pharmaceutical composition comprising a pharmaceutical carrier, and dispersed therein, a therapeutically effective amount of a compound of Claim 5.

25

18. A method for inhibiting prenyl-protein transferase which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 1.

19. A method for inhibiting prenyl-protein transferase which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 3.
- 5
20. A method for inhibiting prenyl-protein transferase which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 5.
- 10
21. A method for treating cancer which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 1.
- 15
22. A method for treating cancer which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 3.
- 20
23. A method for treating cancer which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 5.
- 25
24. A method for treating neurofibromin benign proliferative disorder which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 1.
- 25
25. A method for treating blindness related to retinal vascularization which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 1.
- 30
26. A method for treating infections from hepatitis delta and related viruses which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 1.

27. A method for preventing restenosis which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 1.

5 28. A method for treating polycystic kidney disease which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 1.

10 29. A method of conferring radiation sensitivity on a tumor cell using a therapeutically effective amount of a compound of Claim 1 in combination with radiation therapy.

15 30. A method of using a therapeutically effective amount of a compound of Claim 1 in combination with an antineoplastic to treat cancer.

 31. A method according to Claim 22 wherein the antineoplastic is paclitaxel.

20 32. A pharmaceutical composition made by combining the compound of Claim 1 and a pharmaceutically acceptable carrier.

25 33. A process for making a pharmaceutical composition comprising combining a compound of Claim 1 and a pharmaceutically acceptable carrier.

SEQUENCE LISTING

<110> Merck & Co., Inc.
 Trotter, B. Wesley
 Quigley, Amy G.
 Dinsmore, Christopher J.
 Lumma, William C., Jr.
 Sisko, John T.

<120> Inhibitors of Prenyl-Protein Transferase

<130> 20731

<150> 60/237,241
 <151> 2000-10-02

<160> 25

<170> FastSEQ for Windows Version 4.0

<210> 1
 <211> 4
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Completely synthetic sequence

<400> 1
 Cys Val Leu Leu
 1

<210> 2
 <211> 4
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Completely synthetic sequence

<400> 2
 Cys Val Leu Ser
 1

<210> 3
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Completely synthetic sequence

<400> 3
 Gly Lys Lys Lys Lys Lys Ser Lys Thr Lys Cys Val Ile Met
 1 5 10 15

<210> 4

<211> 52
<212> DNA
<213> Artificial Sequence

<220>
<223> Completely synthetic sequence

<400> 4
gagaggggaat tcggggccctt cctgcatgct gctgctgctg ctgctgctgg gc

52

<210> 5
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> Completely synthetic sequence

<400> 5
gagagagctc gaggttaacc cgggtgcgcg gcgtcggtgg t

41

<210> 6
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Completely synthetic sequence

<400> 6
gagagagtct agagttaacc cgtggtcccc gcgttgcttc ct

42

<210> 7
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> Completely synthetic sequence

<400> 7
gaagaggaag cttggtaccg ccaactgggct gtaggtggtg gct

43

<210> 8
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Completely synthetic sequence

<400> 8
ggcagagctc gtttagtgaa ccgtcag

27

<210> 9
<211> 27
<212> DNA
<213> Artificial Sequence

<220>

<223> Completely synthetic sequence

<400> 9

gagagatctc aaggacggtg actgcag

27

<210> 10

<211> 86

<212> DNA

<213> Artificial Sequence

<220>

<223> Completely synthetic sequence

<400> 10

tctcctcgag gccaccatgg ggagtagcaa gagcaagcct aaggacccca gccagcgccg
gatgacagaa tacaagcttg tggtagg

60

86

<210> 11

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Completely synthetic sequence

<400> 11

cacatctaga tcaggacagc acagacttgc agc

33

<210> 12

<211> 41

<212> DNA

<213> Artificial Sequence

<220>

<223> Completely synthetic sequence

<400> 12

tctcctcgag gccaccatga cagaatacaa gcttgtggtg g

41

<210> 13

<211> 38

<212> DNA

<213> Artificial Sequence

<220>

<223> Completely synthetic sequence

<400> 13

cactctagac tgggtgtcaga gcagcacaca cttgcagc

38

<210> 14

<211> 38

<212> DNA

<213> Artificial Sequence

<220>

<223> Completely synthetic sequence

<400> 14
gagagaattc gccaccatga cggaatataa gctggtgg 38

<210> 15
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Completely synthetic sequence

<400> 15
gagagtcgac gcgtcaggag agcacacact tgc 33

<210> 16
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Completely synthetic sequence

<400> 16
ccgccggcct ggaggagtac ag 22

<210> 17
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Completely synthetic sequence

<400> 17
gagagaattc gccaccatga ctgagtacaa actggtgg 38

<210> 18
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Completely synthetic sequence

<400> 18
gagagtcgac ttgttacatc accacacatg gc 32

<210> 19
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Completely synthetic sequence

<400> 19
gttgagcag ttggtgttg g 21

<210> 20

<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Completely synthetic sequence

<400> 20
gagaggtacc gccacatga ctgaatataa acttgtgg

38

<210> 21
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Completely synthetic sequence

<400> 21
ctctgtcgac gtatttacat aattacacac ttgtgc

36

<210> 22
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Completely synthetic sequence

<400> 22
gtagttggag ctgttggcgt aggc

24

<210> 23
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Completely synthetic sequence

<400> 23
gagaggtacc gccacatga ctgaatataa acttgtgg

38

<210> 24
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Completely synthetic sequence

<400> 24
ctctgtcgac agattacatt ataatgcatt ttttaatttt cacac

45

<210> 25
<211> 24
<212> DNA
<213> Artificial Sequence

<220>

<223> Completely synthetic sequence

<400> 25

gtagttggag ctggtggcgt aggc

24

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/42389

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07D 209/12; A61K 31/4045

US CL : 514/339, 419; 546/277.4; 548/492,494

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/339, 419; 546/277.4; 548/492,494

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,877,202 A (BITONTI et al) 02 March 1999 (02.03.1999), see the formula in col. 1 and the Examples.	1-4, 15-16, 18-19, 21-22, 24-33

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"

document member of the same patent family

Date of the actual completion of the international search

17 December 2001 (17.12.2001)

Date of mailing of the international search report

23 JAN 2002

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703)305-3230

Authorized officer

Deepak Rao

Telephone No. (703) 308-1235

This Page Blank (uspto)

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☒ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.

This Page Blank (uspto)